ACTA PHYSIOLOGICA LATINO AMERICANA

FISIOLOGÍA, FARMACOLOGÍA, BIOQUÍMICA Y CIENCIAS AFINES

VOLUMEN X — 1960 — Nº 1

(Número de homenaje a la memoria de Eduardo Braun Menéndez)

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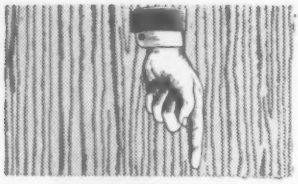

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Eduardo Braun Menéndez

In memoriam

El 16 de enero se cumplió el primer aniversario de la muerte de Eduardo Braun Menéndez, ocurrida en el desgraciado desastre de aviación de Mar del Plata, en el cual perdió también la vida su hija Magdalena.

El año transcurrido no ha amortiguado la intensa sensación de dolor que produjo su inesperado fallecimiento. Muy por el contrario, sólo ha servido para hacerla más nítida y más profunda, porque a cada instante se nos hace patente la noción de lo mucho que perdimos al desaparecer uno de los más eminentes líderes de nuestro adelanto universitario y científico. Se mantiene vivo el sentimiento de pesar producido por la pérdida de este amigo a quien teníamos un profundo afecto y en cuya capacidad y acción entusiasta y clarividente se depositaban tantas esperanzas para el adelanto de la ciencia y de la Universidad en nuestro país.

Gozaba del respeto y la simpatía general, aún de los que discrepaban con él. A poco de conocerlo despertaba afecto y admiración por sus múltiples cualidades, la nobleza de sus ideas, su desinterés y su idealismo. Los discípulos hallaban en él a un maestro de espíritu cordial, atrayente, ansioso de comprenderlos y ayudarlos. Por eso, al perderlo se sintieron desamparados y atónitos por el tremendo golpe inesperado. Atraía a muchos jóvenes y estaba comenzando a formar una escuela, la cual tiene la obligación moral de proseguir su obra y sus orientaciones.

Era franco en expresar sus pensamientos, pero cuando contradecía procuraba no herir. Fué modesto porque amaba a sus ideales, a la ciencia y a la Universidad más que a la fama y los honores, que le fueron otorgados merecidamente sin que los buscara.

Mostró firme lealtad a sus principios, a sus maestros y a sus amigos. Dió prueba de ello en momentos muy difíciles, sin vacilar y sin ostentación.

Todo lo humano le interesaba y tenía amplia cultura general, científica y artística. Era un hombre dotado en forma extraordinaria, física, intelectual y moralmente.

Dinámico, emprendedor, optimista y luchador infatigable, colaboró en todas las instituciones de fomento de la ciencia, de formación de investigadores y de ayuda a su obra, como ser: la Asociación Argentina para el Progreso de las Ciencias, el Consejo Nacional de Investigaciones Científicas y Técnicas, la Sociedad Científica Argentina, que presidía. En épocas difíciles fundó el Instituto

Católico de Ciencias que en un momento fué la única tribuna académica libre en el país.

Para difundir la ciencia en nuestro país fué uno de los que crearon "Ciencia e Investigación". Fundó "Acta Physiologica Latinoamericana" para que fuera el órgano que reuniera la obra científica latinoamericana en el campo de las Ciencias Fisiológicas y para que la estimulara y la difundiera entre todos los científicos del mundo. Esta obra difícil, que inició hace nueve años, ha tenido éxito y esta revista es hoy el órgano oficial de la ASOCIACIÓN LATINOAMERICANA DE CIENCIAS FISIOLÓGICAS.

Luchó para que nuestra Universidad fuera más moderna y mejor. No escatimó esfuerzos en conferencias, reuniones de educación, mesas redondas, en todas partes, para propagar las buenas orientaciones. Su voz era muy respetada y escuchada. No hay duda de que la Universidad perdió con su muerte a uno de sus espíritus rectores y que más necesitaba.

Tenía fe en el papel de la ciencia para adelantar los conocimientos y la consideraba importante, no sólo para el progreso material y la prosperidad general, sino también para la elevación intelectual y moral.

Su obra científica efectuada durante casi treinta años ha sido original, fecunda y de alta calidad. Lo hizo considerar como una autoridad en el campo de la hipertensión experimental, la angiotensina, los ruidos del corazón, la función renal. Su nombre está vinculado a descubrimientos que han marcado pasos decisivos en el adelanto de la Fisiología y de la Medicina Experimental.

Adquirió un gran prestigio por sus trabajos y por eso era invitado continuamente a Congresos y Simposios. Era además miembro de jurados de la Ciba Foundation. Contribuyó a aumentar las relaciones científicas internacionales. Conquistaba admiradores y amigos en todo el mundo y por eso su muerte produjo un duelo universal.

Grandes actos de homenaje se han celebrado en su memoria por las instituciones científicas y universitarias de Santiago de Chile, Sao Paulo, Río de Janeiro, Estados Unidos de Norteamérica y numerosas instituciones argentinas. Entre ellas, el gran acto celebrado el 14 de mayo de 1959 en Buenos Aires, por la Academia Nacional de Medicina; la Academia Nacional de Ciencias Exactas, Físicas y Naturales; el Consejo Nacional de Investigaciones Científicas y Técnicas; la Asociación Médica Argentina; la Sociedad Científica Argentina; la Sociedad Argentina de Biología; la Sociedad Argentina de Cardiología; la Sociedad Argentina de Fisiología; el Instituto de Biología y Medicina Experimental.

Durante el XXI Congreso Internacional de Ciencias Fisiológicas, cuyo Comité Organizador presidía, se celebró un acto solemne en el que el Profesor Corneille Heymans, presidente de la Unión Internacional de Ciencias Fisiológicas, pronunció la primera conferencia de la "Fundación Eduardo Braun-Menéndez" instituída por la Sra. Herminia Menéndez Behety de Gómez. En el mismo acto, el Presidente de la American Physiological Society entregó a la Señora María Teresa Cantilo de Braun Menéndez, su esposa, el diploma de Miembro Honorario de la misma, entregado por excepción después de la muerte, porque la Sociedad deseaba que su nombre figurara en el elenco de sus miembros y porque su designación había sido ya propuesta antes de su fallecimiento.

El recuerdo indeleble de Eduardo Braun Menéndez inspirará, estimulará y guiará a los que dedican su actividad al adelanto de las Ciencias Fisiológicas.

Esta revista se honra en tributar este modesto homenaje de recuerdo al hombre eminente que fué su fundador, inspirador y director.

B. A. HOUSSAY

Nuestros amigos forman parte de nuestra vida y nuestra vida explica nuestras amistades.

(RAISSA MARITAIN)

Escribir un artículo científico, narrar las impresiones de un viaje, comentar la situación universitaria o política, son tareas que requieren un cierto esfuerzo y método; la práctica, sin embargo, da una cierta facilidad para realizarlos.

Mas, es muy difícil escribir sobre un amigo. Por lo general no se escriben artículos sobre los miembros de la propia familia y no hay duda de que un amigo forma parte de la familia espiritual que vamos formando durante nuestra vida. Y a propósito, ¿qué es un amigo? "Aquél que es capaz de dar la vida por el amigo" reza la definición evangélica que Braun-Menéndez cumplió durante su vida y selló con su muerte accidentada, en la cual le acompañó su hija Magdalena.

Los recuerdos se amontonan desordenadamente haciendo presión sobre los puntos de la pluma que sólo los deja pasar uno a uno, como en un estrecho desfiladero.

El primero que aparece es el de aquella mañana en que tímidamente me acerqué al Profesor Houssay a fin de manifestarle mi deseo de trabajar en investigación. Recorrimos varios laboratorios del departamento de fisiología de la vieja Facultad de Medicina, fríos y oscuros, como corresponde en general, no sé por qué razón, a los antiguos laboratorios. Llegamos finalmente a uno en que se trabajaba sobre una preparación cardio-pulmo-renal. Quien la realizaba era un hombre joven, recién regresado de Inglaterra donde había estudiado con el Profesor Lovatt Evans. Porte elegante, atlético y distinguido. Mirada franca y apretón de manos también franco. Manifesté al Profesor Houssay mi deseo de adscribirme a ese laboratorio y ayudar al Dr. Braun-Menéndez. ¿Fué la preparación cardio-pulmo-renal el motivo de tal preferencia o fué el hombre? Creo que fué el hombre. Y no me equivoqué. La salud espiritual trasciende, forma una atmósfera alrededor de quien la posee; no se expresa en una fórmula matemática y no tiene un sistema objetivo de medida. Pero el alma que la posee, la expresa y un observador profundo la mide. Braun-Menéndez la poseía a raudales y la volcaba sobre aquellos que se acercaban a él. Es curioso, pero, una vez llegados a la madurez, al lanzar una mirada retrospectiva, observamos que no quedan en el recuerdo aquellos que nos enseñaron una técnica o nos indicaron un método, sino aquellos que, por su jerarquía espiritual, imprimieron un carácter en el nuestro; los que por su rectitud, bondad y desprendimiento nos enseñaron a ser rectos, bon-

dadosos y desprendidos; los que por su salud espiritual sirvieron de médicos para nuestro espíritu quizás sin saberlo. Braun-Menéndez perteneció a esta categoría de hombres.

El forzado abandono de la Facultad de Medicina llevó parte del grupo del Profesor Houssay a trabajar en un lugar pequeño, que rápidamente se transformó en uno de los focos de luz en aquellos años de oscuridad nacional. La limitación espacial suele tener una ventaja, pues impone un mayor contacto humano y en consecuencia un conocimiento más íntimo, hecho que no sucede en los institutos exageradamente grandes. Pero a su vez el conocimiento íntimo de un hombre, a quien se admiró a distancia. depara comúnmente la traumatizante sorpresa de un desengaño. El conocimiento íntimo puede ser lo que el fuego para la cera: derrite los falsos valores. Pero también puede ser como el buril para la roca: pone en evidencia más virtudes en el valor auténtico. Estos últimos no temen la intimidad. Braun-Menéndez no la temía; más aún, la procuraba. Escribí en una oportunidad y lo repito aquí: "El que dá, no puede dar a distancia; sólo el mezquino o el soberbiamente pequeño se mantiene distante. La donación exige negación de sí mismo, pues el conocimiento de nosotros por los demás, tal cual somos, sin aparentar ni simular nada, tiene como base una auténtica sencillez de espíritu no fácil de alcanzar". No sé en quién pensaba cuando en 1955 escribí lo transcripto, pero ahora puedo afirmar que su recuerdo fué evocado por la auténtica sencillez de espíritu que animaba a Braun-Menéndez.

Con su entusiasmo habitual solía decir:

"Si la gente se apercibiera de los beneficios prácticos que trae el ser bueno y honesto, todo el mundo sería bueno y honesto, no por virtuoso, sino por la utilidad material que le reporta."

Su excesiva bondad, su rectitud de propósitos le llevaron a menudo a equivocarse con respecto a personas y a ser engañado, pues no podía suponer que quien le hablaba podría albergar sentimientos y pensamientos contrarios a aquellos que manifestaba. Esta virtud fué aparentemente una desventaja en algunos ambientes en que tuvo que actuar.

Su catolicismo bien vivido le permitió desarrollar una personalidad integral, armónica, sin esas hipertrofias o atrofias tan comunes en hombres de laboratorio que no poseen una filosofía que les permita combatir esas deformaciones, a no ser aquella que ellos mismos crean para justificarlas. La ciencia no constituyó su Dios; fué solamente un medio para realizarse y mejor servir a los demás. No había rasgos en su persona indicadores de que su actividad era motivada por el deseo de gloria y fama, fuente de estímulos para muchos y también de egoísmos y pequeñeces, pero ellas le llegaron por "añadidura" y como tal no le deformaron pues fueron meros accidentes que nunca lograron integrar la esencia de su modo de ser.

Sus intereses intelectuales eran amplios: filosofía, religión, literatura y artes en general. Era un excelente pianista; confesó en una oportunidad que en determinado momento de su vida tuvo que decidirse entre la investigación científica y la carrera de concertista. No obstante, sus sueños de concertista no quedaron frustrados. No tuvo para sus ejecuciones el público inquieto y nervioso que llena las plateas de un teatro, sino el público íntimamente hogareño, donde su placer de padre rebosaba en su sonrisa amplia al ser acompañado por alguna de sus hijas.

No faltó el deporte entre las facetas que integraban su personalidad, tan plena de entusiasmo vital. Polista en su juventud, golfista en su madurez, seguía de cerca todas las actividades de-

portivas. Recuerdo que en más de una ocasión fuí compañero de "escapadas" del laboratorio a fin de asistir a partidos de polo, en los cuales dos de sus hijos integraban en forma eficiente uno de los equipos. O bien era un partido de tennis en el que intervenía un pariente que adquirió categoría internacional.

Su personalidad científica ha sido vastamente comentada y eso me exime de hacerlo y caer en repeticiones. En esta misma revista el Dr. Lewis escribió un excelente artículo.

La última etapa de su actividad universitaria fué cumplida allí donde la había iniciado, es decir, en la Facultad de Medicina de Buenos Aires. A ella brindó, como en todo, su energía y entusiasmo sin reservas, sacrificando su trabajo de laboratorio que era su vocación, para lanzarse al siempre pesado y no siempre gratificante trabajo de organización. Un día me dió cita en la Facultad a las 0,30; allí lo encontré tomando examen, actividad que había desarrollado todo el día, como así también durante muchos días anteriores y con la dramática perspectiva de continuarla muchos días más. Esa actividad monstruosa, anti-intelectual, le agotaba. Pero había puesto la mano en el arado y no lo abandonaría, pues tenía conciencia de que era lo que le correspondía desarrollar en esa etapa de su vida. En un medio universitario de aguas no muy limpias por la intromisión de intereses extrauniversitarios, fué resistido y combatido. Pero cuando la ignorancia dió lugar al conocimiento de su personalidad y los preconceptos a la razón, la juventud estudiantil, cansada de falsos profetas, se apercibió de que había encontrado un guía auténtico. Cuando comenzaron a conocerle, comenzaron a amarle y a seguirle. Fué el momento de su desaparición. La juventud universitaria le lloró sinceramente. El mundo científico hizo un minuto de silencio. Su familia espiritual, desolada, recordó las palabras del Eclesiástico:

> *"El amigo fiel es una protección fuerte y el que le ha encontrado, ha encontrado un tesoro."*

MIGUEL R. COVIÁN

SEXUAL HORMONES AND RENAL HYPERTENSION

E. Braun Menéndez * and J. C. Penhos
(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires.)

In previous works, the effect of different hormones on the blood pressure of rats with reduced renal mass was studied, starting from the hypothesis that, when the kidney is unable to respond to an increase of "renotrophin" with an increase of its own mass, hypertension is observed (Braun-Menéndez, 1952). Thus it was demonstrated in rats with reduced renal mass that a hypertensive effect was produced by thyroid powder administration or thyroxine (Braun Menéndez, 1954), somatotrophin (Braun Menéndez and Penhos, 1955 a), thyrotrophin (Braun Menéndez and Penhos, 1955 b), adrenocorticotrophin (Braun Menéndez and Penhos, 1956) and chorionic and serum gonadotrophins given together (Braun Menéndez et al, 1955). The results obtained with the latter led the authors to study the role of sex, testosterone and estradiol, alone or in combination with chorionic and serum gonadotrophins.

Material and Methods

White rats of the Institute strain were used, fed the usual diet (milk: 64 %; bread: 20 %; wheat flour: 8 %; corn: 8 %; with a periodical supplement of cod liver oil, alfalfa and yeast). When the animals reached 95 to 110 g body weight, ligature in 8 of the left kidney was performed following Grollman's technique (1944). Fifteen days later, the right kidney was removed. The technique of Williams et al. (1939) slightly modified, was used to measure blood pressure every 15 days during 3 months and then, after the beginning of hormone treatment in the 4th month, every 4 days.

Hormones, in 0.9 % saline solution, were injected subcutaneously for 20 days, at the following doses: testosterone propionate (microcrystals), 1mg/rat/day; estradiol benzoate (microcrystals), 10 μg/rat/day; chorionic and serum gonadotrophins, simultaneously, 5 U of each, i.e., 10 U/rat/day. The control animals were given 0.5 ml of a 0.9 % saline solution. Castration was performed at the same time as the ligature of the left kidney.

All the animals were given a 1 % NaCl solution to drink *ad libitum*. Body weight was checked on the days of blood pressure determinations.

(•) Deceased January 16, 1959.

TABLE I

White rats with initial body weight 95 to 110 g, ligature of left kidney and right nephrectomy, treated with hormones subcutaneously during 20 days. Blood pressure was checked every 15 days during 3 months before hormone treatment and then every 4 days during treatment. Final body weight was 297 ± 44 g for the females and 266 ± 39 g (*) for the males.

	Number of hypertensive rats			
	males	**castrated males**	**females**	**spayed females**
Controls	4/10	2/10	2/10	1/9
Testosterone 1 mg/rat/day	10/10	9/9	8/10	8/9
Estradiol 10 µg/rat/day	3/9	1/10	0/8	0/10
Serum and Chorionic Gonadotrophins 10 µg/rat/day	8/10	2/9	1/9	1/10
Ser. and Chor. Gonadot. + Testosterone	9/9	10/10	5/10	8/8
Ser. and Chor. Gonadot. + Estradiol	7/10	3/10	0/10	1/10

(*) Standard deviation.

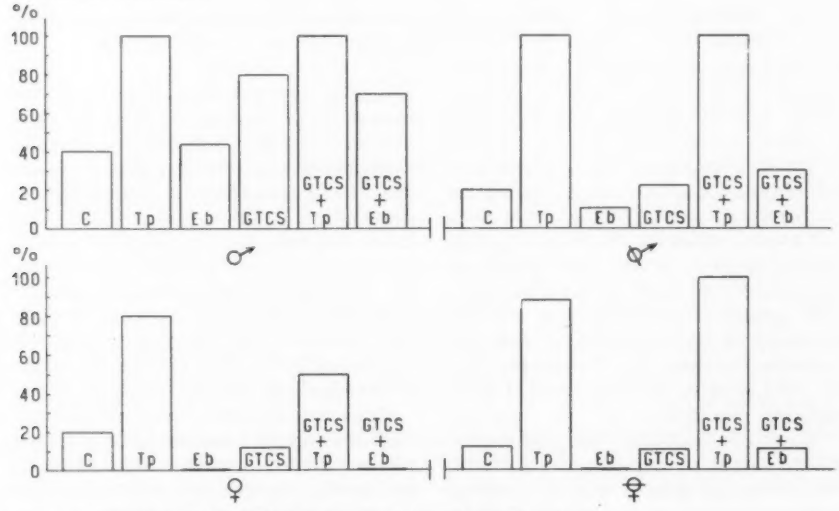

FIG. 1. — *Percentage of hypertensive rats in different groups studied*
C = control animals
TP = treated with testosterone propionate
EB = treated with estradiol benzoate
GTCS = treated with serum and chorionic gonadotrophins.

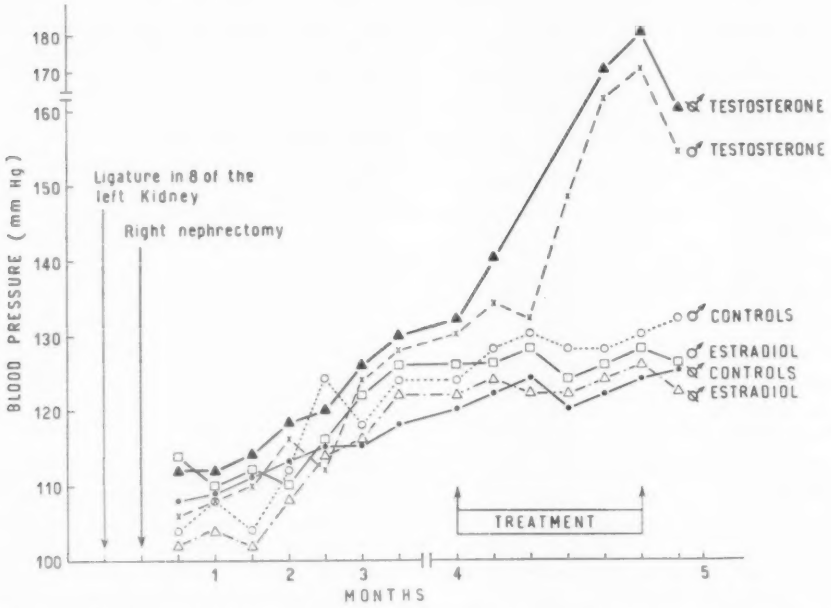

FIG. 2. — *Blood pressure (average) of male rats (intact and castrated) with renal mass reduced, treated during 20 days with testosterone propionate and estradiol benzoate.*

RESULTS

Table I represents the number of hypertensive rats in each group. Testosterone propionate, given alone, produced hypertension in all the groups studied: 100 % in males, intact and castrated; 80 and 89 % in females, intact and spayed respectively (fig. 1). The intensity of hypertension in male rats receiving testosterone, was nearly similar, reaching a maximum of 170-180 mm Hg (fig. 2).

Estradiol benzoate did not modify the incidence of hypertension in either of the studied groups (fig. 1) although both the intact and the castrated males, treated with estradiol, showed lower values than the respective groups of controls.

In female rats (fig. 3) it was again observed that the maximum values of hypertension, oscillating between 154 and 164 mm Hg, belonged to testosterone propionate treated rats. Conversely, those treated with estradiol benzoate showed lower values than the saline treated controls. Gonadotrophins given in conjunction with testosterone propionate produced a hypertensive effect in all the treated groups but of lower intensity in non-spayed females (fig. 1), effects being similar to those obtained with testosterone alone.

Estradiol benzoate, when added to gonadotrophins did not modify the effects of the latter hormones. Castrated males and both intact and spayed females gave a low percentage of hypertensives.

Gonadotrophins had a marked effect only in intact males, the blood pressure reaching a maximum value of 162 mm Hg (fig. 4).

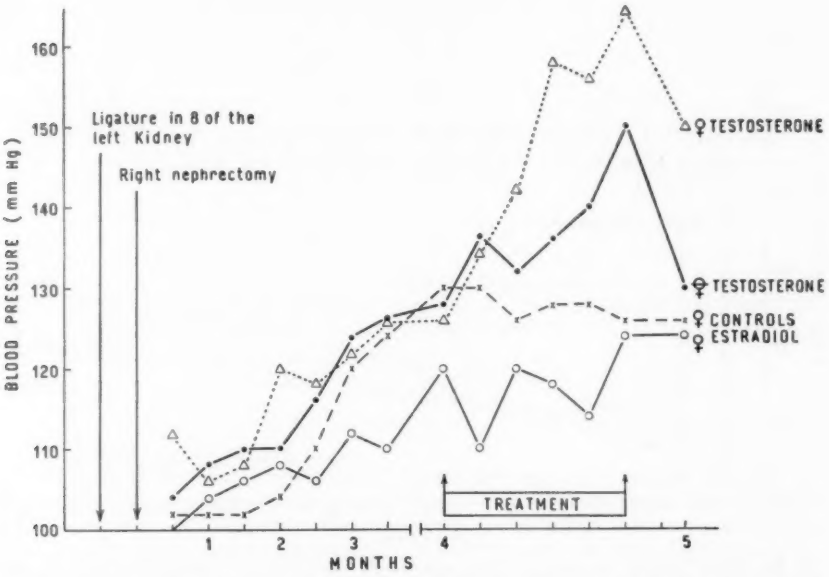

FIG. 3.—*Blood pressure (average) of female rats (intact or spayed) with renal mass reduced, treated during 20 days with testosterone propionate or estradiol benzoate.*

DISCUSSION

The results described show evidence for the hypertensive effect of testosterone propionate in rats with unilateral nephrectomy and ligature in 8 of the remaining kidney. This effect is observed in animals of both sexes, both castrated and intact.

Furthermore, estradiol benzoate had no effect alone and when injected in combination with gonadotrophins did not show a significant difference from the group receiving gonadotrophins alone.

In intact males, a high percentage of hypertensive animals is observed with gonadotrophin treatment (Braun Menéndez et al., 1955) while in castrated males or in females, intact or spayed, the same treatment does not modify the results observed in the controls. Evidently, castration in the males under treatment with gonadotrophins reduces the number of hypertensive animals. This effect of gonadotrophins is described by Sturkie and Ringer (1955) in the chicken, where suppression of gonadotrophin secretion due to diethylstilbestrol administration provoked a fall of the blood pressure in intact and castrated males, whose normal pressure is 15-12% higher than the females. Conversely, the injection of pregnant mare serum containing gonadotrophins increased the blood pressure level in males, castrated and intact, after the pressure was lowered by diethylstilbestrol which had no effect on the blood pressure of normal females. Perry (1955) notes the appearance of periarteritis nodosa in male rats receiving gonadotrophins (Follicle Stimulating Hormone), a phenomenon also observed by the authors of the present paper (Braun Menéndez et al., 1955).

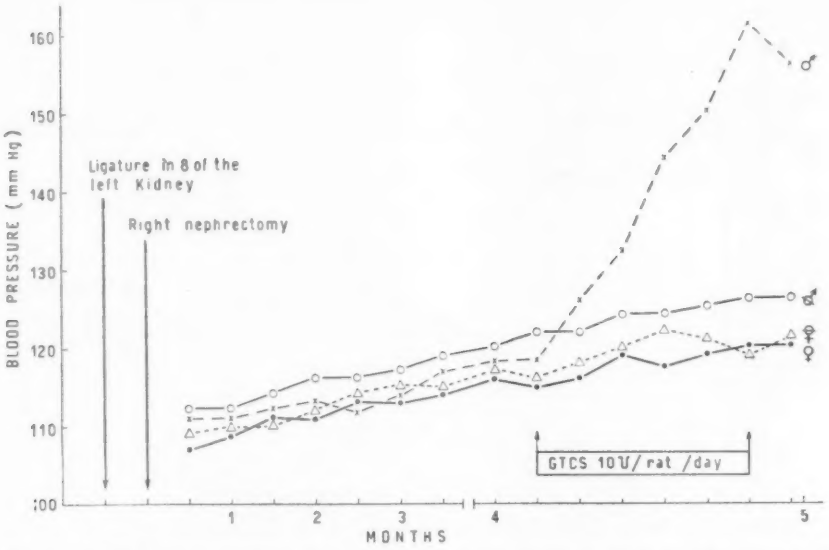

FIG. 4. — *Blood pressure (average) of male and female rats (intact or castrated) with renal mass reduced, treated during 20 days with a combination of serum and chorionic gonadotrophins.*

Our experiments clearly show that the hypertensive effect of gonadotrophins is indirect and produced by gonadal stimulation in the male, increasing in this way their normal level of hormonal secretion. Castration annuls this effect.

SUMMARY

Testosterone propionate has a hypertensive effect in male and female rats with unilateral nephrectomy and ligature in 8 of the remaining kidney, both castrated and intact.

Serum and chorionic gonadotrophins increase the number of hypertensive animals in male rats; this effect is not observed after castration.

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A STUDY ON EARLY POST-NEPHRECTOMY CHANGES OF THE REMAINING KIDNEY IN DOGS *

MARCELINO CEREIJIDO
(Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad de Buenos Aires.)

IN 1952 Braun Menéndez put forward a hypothesis on renal arterial hypertension stemming from earlier observation on compensatory renal hypertrophy (4). He assumed the existence of one or more factors which would normally stimulate kidney growth ("renotrophin"); he then postulated a "normal" ratio between renotrophin and kidney mass. Alteration of this ratio, whether by a reduction of kidney mass or by increasing renotrophic factors without a corresponding renal growth, would determine an increase in arterial pressure.

In order to obtain further data on kidney growth, observations during the first twenty days after unilateral nephrectomy are reported. We have studied this period because it is here that marked changes in kidney mass do occur.

MATERIAL AND METHODS

Female trained mongrel dogs, weighing between 6.5 and 12.5 kg, maintained on beef heart and water *ad libitum*, were used. Before tests, the animals were given water but no food for at least 12 hours. One hour before starting the experiments, the dogs were hydrated by stomach tube in amounts ranging from 6 % to 8 % of body weight. During the tests they stood on a table gently held by two leather straps. Glomerular filtration was determined by clearance of inulin [Roe, Epstein and Goldstein's method as described by Schreiner (19] and renal plasma flow by p-aminohippurate [Bratton and Marshall's method (3) as modified by Smith, 1945 (20)]. After the clearances were determined, the arterial blood pressure was measured by connecting the femoral artery to a mercury manometer through a 1.0 mm O.D. needle and blood was extracted to obtain the hematocrit by duplicate.

A posterior left nephrectomy was made within three days of the control clearance determinations. The same determinations were then repeated in groups of six animals 5, 10, 15 or 20 days after the operation, when the dogs were sacrificed and the right kidney excised. The kidneys were decapsulated and carefully expressed with a gauze;

(*) These experiments were partly supported with a grant from the "Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina".

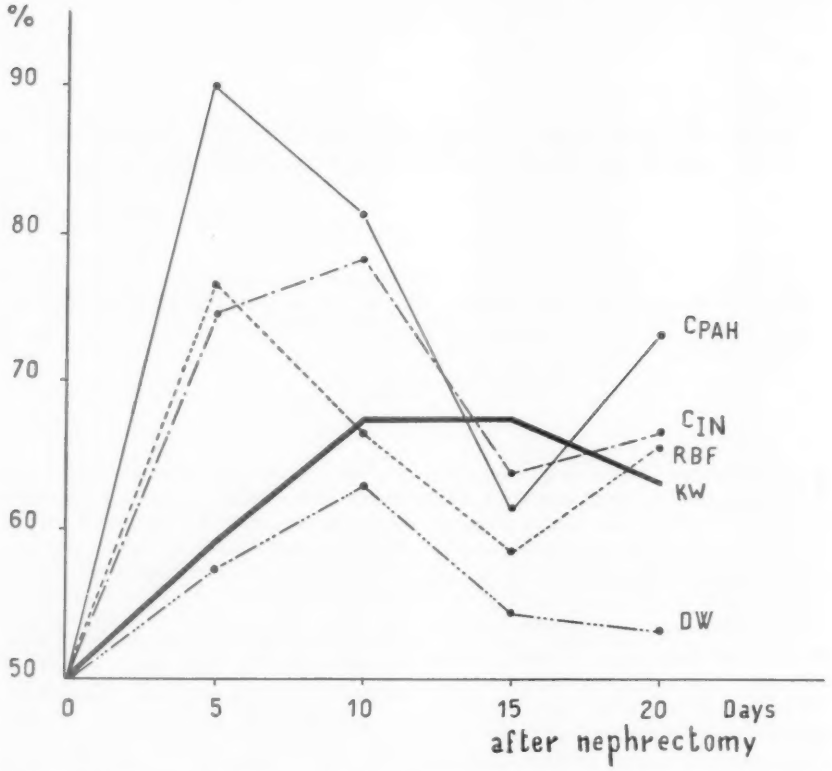

FIG. 1.—*Kidney weight and clearances of remaining kidney in uni-nephrectomized dogs. (Expressed as percent of control values.) For dispersion of values, see Table III. KW, kidney weight; DW, dry kidney weight.*

they were then cut into pieces and placed on glass capsules up to 100°C until constant weight was reached. Twice the weight of the first removed left kidney was considered to be the dog's "total kidney mass". The theoretical change in kidney weight which could have taken place between the observations, was considered to be slight and, consequently, of no significance.

Table I shows results obtained in six dogs with the kidneys tested separately under chloralose anesthesia (100 mg/kg). The mean values thus obtained are lower than in animals when awake [Houck 1948 (9)], probably due to the action of anesthesia, surgery, etc. Since the differences here observed between both kidneys were not significant, it has been assumed that the function of each kidney represents 50 % of the total renal function.

The body surface of the animals was calculated with Smith's nomogram according to Cowgill and Drawkin's formula [Smith 1956 (22)]. Results are ex-

pressed as the mean value ± standard error of six animals.

Results

Table II shows normal (pre-nephrectomy) values of the dogs used in these experiments.

Glomerular Filtration: (Table III and fig. 1). The glomerular filtration showed a rapid increase 5 days after nephrectomy, which was still high 10 days after the operation, decreasing slightly until the 20th day of the observation.

Renal plasma flow and renal blood flow: (Table III and fig. 1). The renal plasma flow showed a rapid increase on the 5th and 10th day; it decreased on the 15th and 20th day. Changes in kidney blood flow are not so marked, probably because of a decrease in the hematocrit after nephrectomy.

Clearance/renal weight ratios: (Table IV). In order to compare the degree of functional recovery and compensatory hypertrophy we have studied several clearance/renal weight ratios. For instance, in a kidney whose weight and particular clearance five days after operation were 65 % of the control values, the relation would be $\frac{65}{65}=1$, which indicates a parallel increase in glomerular filtration and weight. Table IV shows that except for the C_{PAH} ($p < 0.01$) and renal blood flow ($p < 0.01$) at the 5th day, the ratio was not significantly different from 1.0.

Wet/dry kidney weight ratio: (Table V). As shown in table V the wet/dry kidney weight ratio increases after unilateral nephrectomy except at the 5th day. As a consequence (see fig. 2), the changes expressed in dry weight are less apparent than those expressed in wet weight. The dry weight was higher on the 5th and 10th (Table VI) than on the 15th and 20 th day; we shall return later to this point:

Arterial pressure: (Table VII). The arterial pressure showed no significant changes during our observations.

Discussion

Several factors should be considered for a correct interpretation of the results :1) the diet, which was made up exclusively of meat, and 2) the age of dogs, which was not known. The ingestion of a protein rich diet increases the kidney function and its weight, in the dog [Moustgaard 1948 (16)] and in the rat [Addis, Mac Kay and Mac Kay 1926 (2), Mac Kay 1928 (12), 1928 (13), 1938 (11), Smith and Moise 1927 (19)]. Furthermore, it is also known that age modifies the intensity, duration and other characteristics of compensatory hypertophy [Mac Kay 1928 (13, 14)], and this may have increased the dispersion of our data.

The immediate increase in clearances, as well as in weight of the kidneys, observed in our experiments, is consistent

TABLE I

Clearances determined separately in both kidneys of dogs.

Number of dogs	C_{IN} ml/kg/Min.		C_{PAH} ml/kg/Min.	
	left	right	left	right
6	1.59 ± 0.20 (*)	1.54 ± 0.22	4.62 ± 0.53	4.63 ± 0.52

(*) In this and the following tables, mean ± standard error.

TABLE II

Data in normal female dogs before uni-nephrectomy.

Number of dogs	24
C_{IN}	89.8 ± 4.42 ml/m²/Min.
C_{PAH}	259.3 ± 13.7 „
Renal blood flow	524.2 ± 29.3 „
"Total kidney mass"	84.6 ± 2.04 g/m²
Dry weight of total kidney mass	17.3 ± 0.72 „

TABLE III

Kidney weight and clearances of remaining kidney in uni-nephrectomized dogs. (Expressed as percent of control values.)

Days after nephrectomy	Kidney wet weight	C_{IN}	C_{PAH}	Renal blood flow
5	59.7 ± 2.02	74.5 ± 9.92	90.0 ± 7.54	75.5 ± 5.80
10	67.5 ± 1.70	79.2 ± 7.45	80.7 ± 12.48	66.9 ± 12.65
15	67.1 ± 4.80	63.5 ± 7.02	62.6 ± 10.10	58.0 ± 12.32
20	62.8 ± 4.49	66.9 ± 5.67	72.5 ± 10.45	66.3 ± 9.38

TABLE IV

Clearance wet weight relative ratios of remaining kidney after unilateral nephrectomy in dogs. C_{IN1}; C_{PAH1}; RBF_1; WW_1: glomerular filtration, renal plasma flow, renal blood flow and wet weight before nephrectomy. C_{IN2}; C_{PAH2}; RBF_2 and WW_2: the same parameters after nephrectomy.

Days after nephrectomy	$\frac{C_{PAH2}/C_{PAH1}}{WW_2/WW_1}$	$\frac{C_{IN2}/C_{IN1}}{WW_2/WW_1}$	$\frac{RBF_2 / RBF_1}{WW_2 / WW_1}$
5	1.67 ± 0.13 (••)	1.25 ± 0.11 (•)	1.26 ± 0.09 (••)
10	1.19 ± 0.17	1.17 ± 0.10	0.99 ± 0.17
15	0.93 ± 0.09	0.95 ± 0.15	0.87 ± 0.18
20	1.15 ± 0.09	1.13 ± 0.09	1.05 ± 0.10

(•) $p < 0.05$
(••) $p < 0.01$

with previous reports [Morel and Verliac 1913 (15)].

The increase in size and function which immediately follows nephrectomy, and the appearance of the organ had led some authors to believe in the past, that the increase in kidney volume was due to an increased retention of urine, blood and lymph [Rosenstein 1871 (18)]. Morel and Verliac pointed out in 1913, that compensatory hypertrophy in dogs was at its highest between the 4th and 10th day, falling between the 10th to the 20th day, to increase later to a definite level (about 80 %). They interpreted only the first increase as a due to congestion and edema. Other authors [Braun Menendez 1946 (5), Rollason 1949 (17)] working with rats, did not observe such decreases in kidney weight although, an increase in C_{IN} and Tm_D was demonstrated to occur following unilateral nephrectomy with a late decrease around the 20th day. [Braun Menéndez and Chiodi 1947 (6)].

A) control. Wet/dry weight ratio	Days after nephrectomy	B) after nephrectomy. Wet/dry weight ratio
5.13 ± 0.11	5	5.30 ± 0.21
4.86 ± 0.20	10	5.30 ± 0.15
4.66 ± 0.24	15	5.79 ± 0.21
4.65 ± 0.26	20	5.50 ± 0.31

TABLE V

Wet/dry weight ratios of kidneys in uni-nephrectomized dogs.

Days after nephrectomy	Dry weight of right kidney (percent of control values)
5	57.2 ± 2.78
10	62.2 ± 3.60
15	54.4 ± 3.59
20	53.9 ± 1.89

TABLE VI

Changes in dry weight of remaining kidney after unilateral nephrectomy.

Days after nephrectomy	Blood pressure in mm Hg	
	before nephrectomy	after nephrectomy
5	131.3 ± 7.80	138.3 ± 4.47
10	132.8 ± 10.85	130.0 ± 2.68
15	133.8 ± 6.94	137.5 ± 6.68
20	121.3 ± 6.04	124.6 ± 6.08

TABLE VII

Arterial blood pressure in dogs.

In 1948 Moustgaard reported that the dog also showed an increase in the inulin and Hippuran clearances which nearly reached the level registered prior to uninephrectomy. These values decreased later, to rise again attaining a stable level (65 % to 75 %) around the second or forth week. These results as

well as those previously described by Braun Menendez and Chiodi in rats, are consistent with those included in this paper.

The fact that the

$$\frac{C_{PAH}}{\text{wet weight}} \quad \text{and} \quad \frac{RBF}{\text{wet weight}}$$

relative ratios (See Table IV) at the 5th day are higher than 1.0, agrees with histological observations [De Robertis and Poch 1947(7), Morel and Verliac 1913 (15), Himman 1926 (8)] reporting a marked congestion and an increased glomerular size.

The wet/dry weight ratio (Table V) increase in kidneys undergoing compensatory hypertrophy indicate that they have a greater total water content. Table VI shows that dry kidney weight first increases, and then decreases at the 15th and 20th days. This indicates not only a water loss, but also a decrease of solid substances. The facts that a considerable proportion of the kidney weight is made up of blood [Swann, Feits and Lowe 1955 (23), Lochner and Ochwadt 1954 (10)], and that in the weighing technique, the kidney is not totally deprived of blood, possibly indicate that changes in the amount of of blood may account for the observed changes in kidney weight. Variations in the cellular and/or the intercellular substances should probably also be taken into consideration.

We acknowledge the assistance of: Dr. J. Ejden, C. Billordo Peres, V. Nahmod and R. Puy and of Mr. Amadeo Cabodevila, who kindly helped us during the performance of these experiments.

Summary

Determinations of C_{IN}, C_{PAH}, renal blood flow and kidney weight were made in dogs before unilateral nephrectomy and 5, 10, 15 or 20 days afterwards.

Glomerular filtration increases rapidly and decreases alterwards. The other explored parameters follow similar patterns.

Possible explanations accounting for these changes are discussed.

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HAIR GROWTH IN HEMIDECORTICATE RATS

MIGUEL R. COVIÁN AND JOSÉ ANTUNES-RODRIGUES
(Department of Physiology, School of Medicine of Ribeirão Preto, Ribeirão Preto, São Paulo, Brasil.)

THE HYPOTHESIS has been advanced that hemidecortication releases the hypothalamus of the rat from some restraining action exerted either by the cerebral cortex or by some subcortical structure. This results in an increase of gonadotrophin secretion by the hypophysis and consequently in an increased activity of the gonads that accounts for a change in the activity of the adrenals (Covián, Migliorini and Tramezzani, 1959). On the other hand, the influence of gonads and adrenals on hair growth has been shown in rats and mice (Houssay, A. B., 1954).

To test still further the aforementioned hypothesis, the growth of hair in hemidecorticate rats was studied.

MATERIAL AND METHODS

Four groups of rats of both sexes, weighing 150-200 g were used. Each group consisted of ten rats and ten sham operated controls. In all groups a close clipping of the back in an area of 6 x 4 cm was made at the moment of operation. The time necessary to restore a normal hair coat was used as a criterion of the rate of hair growth.

First group: Hemidecortication was performed in this group in the usual way.

Second group: Extirpation restricted to the neocortex and archicortex, in order to localize the region responsible of the changes observed, was performed.

Third group: In this group castration was performed simultaneously with partial extirpation.

Fourth group: As hemidecorticate rats lose weight immediately after the operation and regain it later on, a control group of sham operated rats in which a similar change of weight was attained by a restricted diet, was added.

RESULTS

First group (hemidecorticate rats): In hemidecorticate female rats the denuded areas were covered at a slower rate than sham operated animals of the same age and sex. The results obtained are observed in fig. 1 where hemidecorticate rats are represented in stippled areas and controls in white areas. It is seen that hair begins to grow first in control rats, because 14.2 per cent of them are completely covered at 1.5 months and all of them, that is 100 per cent, at 4.5 months. Instead, 18 per cent of hemide-

corticate rats are covered at 2 months and 100 per cent only at 7 months. Figure 2 shows that hair growth in male control rats is closely similar to the control females: at 1.5 months 11.1 per cent are covered and at 4.5 months 100 per cent. Regarding hemidecorticate rats, 10 per cent are covered at 3.5 months and all of them at 7.5 months.

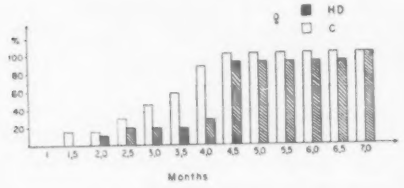

FIG. 1. — *Hair growth in hemidecorticate female rats (stippled columns) and sham operated rats (white columns). The columns represent the percentage of rats fully covered by hair in the area clipped.*

These differences can be seen in fig. 3 and 4. The first one is the photograph of one female hemidecorticate rat (on the left) and its control (on the right) two months after the operation was performed. The control is completely covered while the operated rat is still uncovered. Figure 4 shows the difference between one male hemidecorticated (left) and one control two months after the operation.

Second group: (partial extirpations). The extirpation of the neocortex did not interfere greatly with hair growth as can be seen in fig. 5 and 6. In female rats (fig. 5) at the end of the first month 28 per cent of hemineodecorticated

FIG. 3. — *Hemidecorticate female rat (on left) and control rat after two months of clipping.*

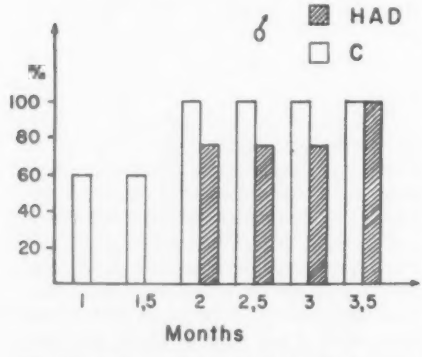

FIG. 2. — *Hair growth in hemidecorticate male rats (stippled columns) and control rats (white columns).*

(stippled areas) and 40 per cent of the controls are covered and at 3.5 months 100 per cent of both are fully covered. Regarding male rats (fig. 6) 50 per cent of the operated rats and 40 per cent of sham operated are covered at the end of the first month and at 2 months the controls, and at two months and a half the operated rats have completed their hair growth.

The extirpation of the archicortex determined a delay in the growing of hair in both sexes. In females (Fig. 7) at the end of the second month 100 per

FE DE ERRATA

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FIG. 2.—*Should be replaced as follows:*

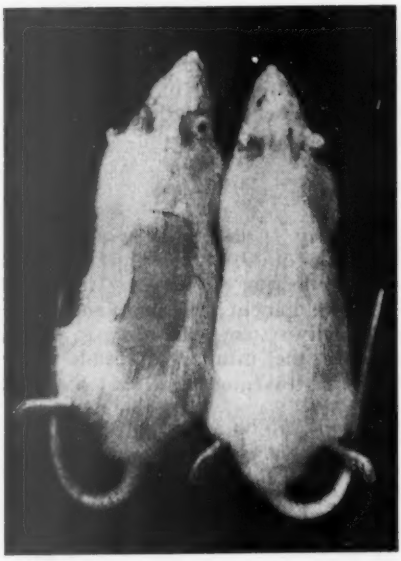

FIG. 4. — *Hemidecorticate male rat (on left) and control after two months of clipping.*

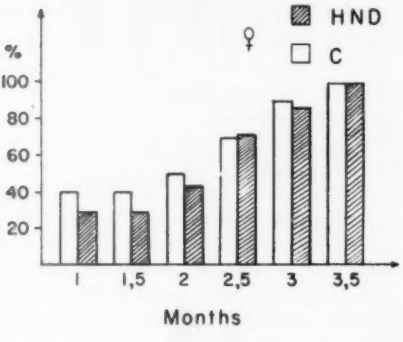

FIG. 5. — *Hair growth in female rats with removal of left neocortex (stippled columns) and control (white columns).*

gure 8 is the photograph of one operated female rat (on left) and one control (on right) after 3 months of the operation. Regarding male rats (fig. 9) at 2 months, 100 per cent of the control rats are covered and only 75 per cent of the operated; this last group reached 100 per cent hair growth at 3.5 months.

Third group: (partial extirpation and castration). In this group the comparison was made amongst rats in which gonadectomy was added to the extirpation of the archicortex and rats with archicortex only removed. Figure 10 represents the results obtained in female rats: it is observed that at one month 10 per cent of the castrated rats (stippled areas) have their clipped zone covered against none of the hemiarchidecorticated; at 2 months the proportion is 50 per cent and 10 per cent respectively; at 3 months, 83 and 60 per

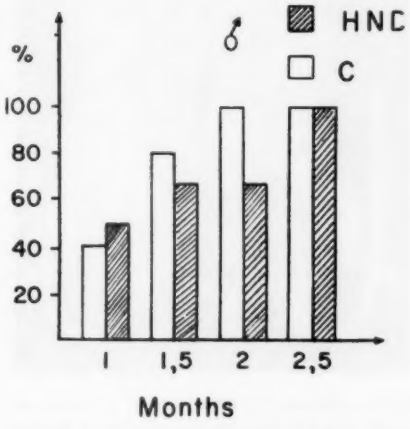

FIG. 6. — *Hair growth in male rats with removal of left neocortex (stippled columns) and control rats (white columns).*

cent of controls (white areas) have their clipped areas fully covered and at the same time only 10 per cent of the operated rats are covered; these ones reached 100 per cent at 4,5 months. Fi- cent; at 4.5 months both groups reach 100 per cent. Regarding males (fig. 11) the sequence is the following: at one month 50 per cent of the rats with combined operation are covered against

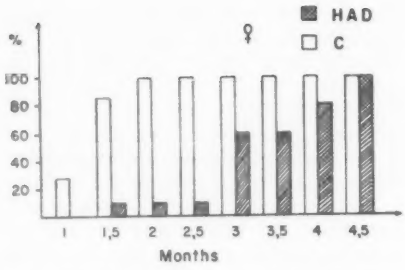

FIG. 7. — *Hair growth in female rats with left archicortex removed (stippled columns) and control rats (white columns).*

FIG. 8. — *Female rat with removal of left archicortex (on left) and control rat after 3 months of clipping.*

none of hemiarchidecorticated; at 1.5 month the same proportion is maintained; at 2.5 months is 80 and 79 per cent respectively; at 3.5 months both groups of rats are fully covered.

Fourth group: The rats, either males or females, in which a loss of weight similar to that of hemidecorticated and hemiarchidecorticated was obtained, did not show any delay in the growth of hair. They behaved as the controls with regular weight.

DISCUSSION

There is experimental evidence that in rabbits, mice, guinea pigs and dogs, some endocrine glands may influence the development and maintenance of the piliary system. According to the literature the pituitary (Freud, 1934; Snow and Whitehead, 1935) and the

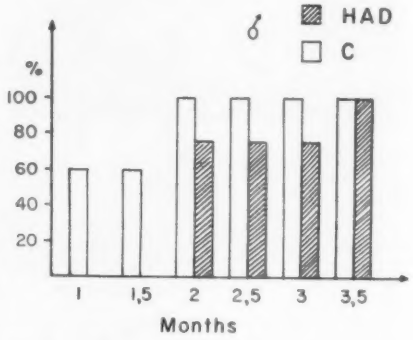

FIG. 9. — *Hair growth in male rats with left archicortex removed (stippled columns) and control rats (white columns).*

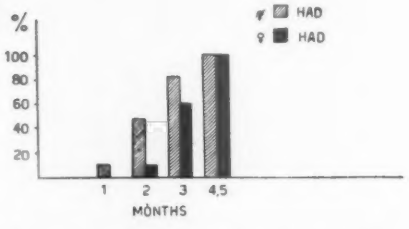

FIG. 10. — *Hair growth in female rats with removal of left archicortex and ovaries (stippled columns) and in rats with archicortex only removed (black columns).*

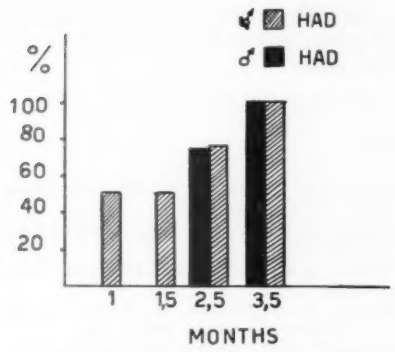

FIG. 11. — *Hair growth in male rats with removal of left archicortex and testes (stippled columns) and in rats with archicortex only removed (black columns).*

thyroid (Chang, 1926; Chang and Feng, 1927; Dieke, 1948) stimulate hair growth in rats and mice; and the adrenals and gonads have an inverse effect. The evidence for the action of sex hormones on hair growth has been studied and the results are rather conflicting. Emmes (1942) and Dieke (1948) found that gonadectomy had no effect on hair growth in either male or female rats. Houssay and Higgins (1949) observed that gonadectomy caused speedier growth over shaved areas in mice of both sexes and Houssay (1953) found that high non physiological doses of sex hormones inhibited hair growth; he also found that the difference of the results of castration between mice (increase in the rate of hair growth) and rats (no change) is due only to the difference in sensitivity of hair follicules to the effects of estrogens and androgens. In a recent publication Houssay, Nallar & Saurer (1959) observed that testosterone propionate in high doses inhibits the hair growth of normal, castrated, hypophysectomized or adrenalectomized rats. Johnson (1958) has shown in rats that gonadectomy does speed the passage of the growth wave but that initiation of the growth wave does not depend upon the presence or absence of gonads in normal rats. It has been also shown that adrenalectomy accelerates the growth of hair in rats (Butcher, 1937; Butcher and Richards, 1939; Ralli and Graef, 1943; Johnson, 1958; Houssay, 1953), and that of all adrenal steroids the C-11 oxygenated are the most effective inhibitors of hair growth.

Hemidecortication in rats leads to a change in the weight of some endocrine glands (Covián, Migliorini and Tramezzani, 1959). Thyroid and thymus are diminished in both males and females; adrenal weight is increased in females and decreased in males. Hemidecorticate male mice show enlarged seminal vesicles and hemidecorticate females show enlarged ovaries. According to these changes it can be argued that the slowing of the hair growth that has been observed in hemidecorticate rats can be attributed to a breakdown with the role played by the different hormones that regulate the piliary system, being the gonads the main factor. This assumption is based upon the results obtained in hemiarchidecorticate-castrated rats. On the other hand, the archicortex seems to be the principal part of the cerebral cortex responsible for the changes mentioned. A general metabolic change in these rats and also a change in sensitivity in hair follicules can not be disregarded.

SUMMARY

Male and female hemidecorticate rats show a delay in hair growth.

Archicortex is the main part of cerebral cortex responsible for this change.

In hemiarchidecorticate-castrated rats hair grows much quicker than in only hemiarchidecorticate rats.

It is advanced that the slowing of the hair growth observed can be attributed to an interference with the role played by the different hormones that regulate the piliary system being the gonads the main factor.

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ANGIOTENSIN, BRADYKININ AND SUBSTANCE "V"

J. C. Fasciolo, K. A. Halvorsen, A. Binia and J. E. Itoiz
(Departamento de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina)

Some highly purified preparations of angiotensin are contaminated by a vasodilator substance [3]. The action of the latter is easily shown when the mixture containing both is injected in the femoral artery of a dog's leg, perfused with a pump, as described elsewhere [2]. The simultaneous recording of the perfusing and systemic pressure shows a fall of the perfusing pressure, produced by the vasodilator substance *substance "V"*, followed after a short delay by a rise in the systemic pressure, due to the angiotensin. This effect is due to the fact that substance *"V"* is rapidly destroyed by blood plasma and most of its activity is lost before reaching the small arterial vessels.

However, if the mixture is injected directly into the aorta, a fall in the systemic pressure is obtained [3, 4].

The vasodilator action of *substance "V"* causes a rapid fall of the perfusing pressure, followed by a slower return to the previous level. The whole effect will last two or more minutes, according to the amount injected.

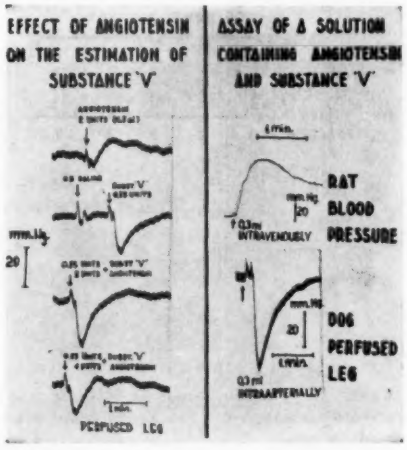
EFFECT OF ANGIOTENSIN ON THE ESTIMATION OF SUBSTANCE 'V'
ASSAY OF A SOLUTION CONTAINING ANGIOTENSIN AND SUBSTANCE 'V'
RAT BLOOD PRESSURE
DOG PERFUSED LEG
PERFUSED LEG

Fig. 1. — Left - Above: *Two Buenos Aires pressor Units of angiotensin in 0.5 ml injected intra-arterially in a perfused leg of a dog were followed by a moderate rise of the perfusing pressure. II) Depressor action of 0.25 Units of substance "V" (0.5 ml volume). III) The depressor action of 0.25 Units of substance "V" is not influenced by adding 2 Units of angiotensin (final volume 0.5 ml).* Below: *4 Units of angiotensin produces little reduction of the depressor effect of 0.25 Units of substance "V".* Right: *0.3 ml of the same solution were injected in the rat (above) and in the perfused leg (below). It can be seen that a pressor effect is obtained in the first case, and a fall of the perfusion pressure, in the second.*

The authors wish to express their appreciation to Dr. M. Rocha e Silva who supplied the bradykinin.

This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

Although angiotensin and *substance "V"* have an opposite action on the vascular tree they can be estimated fairly well in a mixture containing both. The leg preparation has a poor sensitivity to angiotensin and it was found that the addition of different amounts of angiotensin, up to 2 Buenos Aires units (1), to a *substance "V"* dose will not significantly modify the depressor response of the perfused dog's leg to the latter (fig. 1).

On the other hand, angiotensin can be assayed on the blood pressure of the anesthetized rat, in which the pressor effect of angiotensin is predominant, when the mixture is injected intravenously (fig. 1). We suppose that this is due to the fact that the angiotensin injected is destroyed by blood plasma much more slowly than *substance "V"* is.

Separation of angiotensin and substance "V"

Angiotensin preparations in which substance "V" was found, were made as follows (3): a crude renin preparation was incubated for 20 minutes at 37° C with ox globulins, obtained by precipitation of blood plasma with ammonium sulphate between 1.3 and 2.2 M.

The mixture was precipitated with 3 volumes of alcohol, filtered, and concentrated in vacuo. The residue was extracted with ethyl ether, and the water phase extracted several times with butanol. After evaporating the butanol the residue was summitted to a countercurrent distribution in the system 2-butanol 0.1 N HCl, and the content of the tubes with maximum angiotensin activity pooled, evaporated, and the residue extracted with glacial acetic acid.

Since both angiotensin and *substance "V"* are concentrated by the same preparative process which includes a coun-

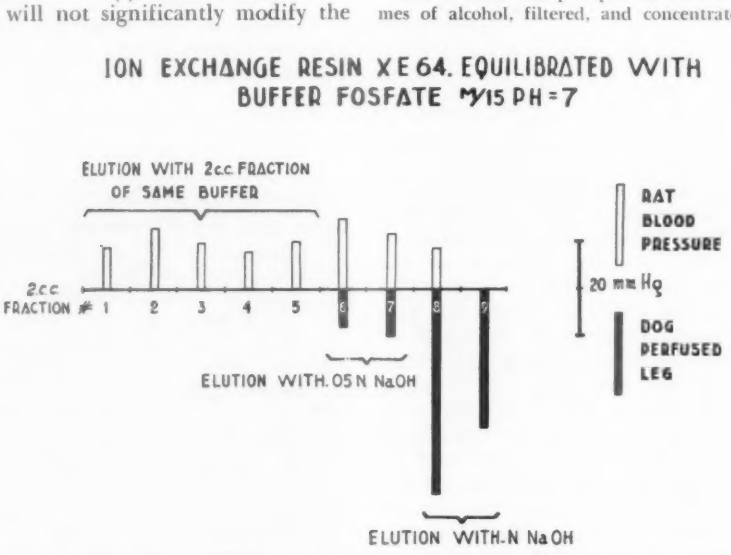
ION EXCHANGE RESIN XE 64. EQUILIBRATED WITH BUFFER FOSFATE M/15 PH = 7
ELUTION WITH 2cc FRACTION OF SAME BUFFER
2cc FRACTION # 1 2 3 4 5 6 7 8 9
ELUTION WITH .05 N NaOH
ELUTION WITH N NaOH
RAT BLOOD PRESSURE
20 mm Hg
DOG PERFUSED LEG

FIG. 2.—*Angiotensin contaminated with Substance "V" was dissolved in distilled water and percolated through a 3 cm XE 64 resin column, previously equilibrated with M/15 phosphate buffer at pH 7. The elution was carried out with five 2 ml fractions of the same buffer, two 2 ml of 0.05 N NaOH and with two 2 ml of N NaOH in that order, and the effluents were injected, in the rat (0.2 ml) (empty columns) and in the perfused dog's leg (0.5 ml) (black columns).*

tercurrent distribution in a system formed by 2-butanol and 0.1 N HCl ([3]) their physicochemical characteristics must be similar. However, we have no quantitative data, about the losses of *substance "V"* during the whole process of preparation.

We have not been able to separate angiotensin from *substance "V"* by paper chromatography, since there has always been an overlapping of the spots, the respective Rf. being very close even if different solvents are used ([3]).

The same results were obtained using paper electrophoresis at various pHs and with different buffers. In many cases both substances overlapped in the same region, although some small areas were free of one or the other ([3]).

The carboxilic ion exchange resin XE 64 (*) equilibrated at pH 7 with phosphate buffer M/15 adsorbs both angiotensin and the *substance "V"*. Binding in the case of angiotensin appears to be slight, since angiotensin could be washed out with the same buffer and eluted with 0.05 M Na OH which eluted little *substance "V"*. On the other hand *substance "V"* is completely eluted, by 1 M Na OH or 0.6 M Na HCO^3. Figure 2 shows the results of a typical experiment.

Comparison of substance "V" with bradykinin

Bradykinin is a polypeptide formed by the interaction of plasma globulins with the venom of *Bothrops jararaca* or with trypsin ([5]); *substance "V"* and bradykinin are both dialyzable through cellophane membranes; they withstand 20 minutes boiling in 0.1 M HCl, but most of their activity is lost by similar treatment in 0.1 M Na OH; both are destroyed by chymotrypsin, tissue extracts and plasma, but not by pepsin ([3]). They cannot be separated by paper chromatrography or paper electrophoresis. A mixture of both substances running in the same strip gives rise to only one spot with vasodepressor activity ([3]).

However a difference can be established by the action of acid on the plasma enzymes which destroy *substance "V"* and bradykinin. Both substances are rapidly destroyed when incubated with blood plasma at pH varying from 7 to 11. If the plasma is acidified at pH 4.5 and incubated at 37° C for 30 minutes, its capacity to destroy bradykinin is irreversibly lost, but its power to destroy *substance "V"* is preserved.

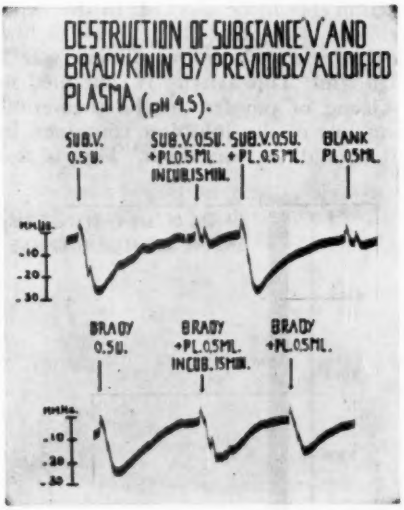

FIG. 3. — *Dog plasma was acidified to pH 4.5 incubated 30 minutes at 37° C and then brought to pH 7.4.* Above: *0.5 Units of substance "V" are completely inactivated by 15 minutes incubation at pH 7.4 with previously acidified plasma. Acidified plasma plus substance "V", without incubation, produces the usual vasodilator effect. Plasma alone produces no effect.* Below: *0.5 Units of bradykinin are not destroyed by incubation with 0.5 ml of previously acidified plasma. Incubation was carried out at pH 7.4.*

(*) Rohm & Haas Co.

Figure 3 shows the results of a typical experiment.

On the other hand the incubation of blood plasma at pH 3.5 for 30 minutes at 37° C inactivates the enzymes which destroy both substances. These results can be explained by assuming that blood plasma has two enzymes: Enzyme I, unstable at pH 4.5 destroys bradykinin and probably *substance "V"*, and enzyme II, which is stable to this pH and destroys *substance "V"* but not bradykinin. On this basis *substance "V"* and bradykinin should be considered different polypeptides. Another explanation could be the presence of an inhibitor of enzyme II in the bradykinin preparation, so that its action should be blocked. In the experiment of figure 3 the amount of bradykinin added to the plasma was 1 Unit/ml. This activity is contained in 0.1 mg of powder, so the theoretical amount of the inhibitor contained in it should be very high. This is not likely, considering the method used to prepare bradykinin. For this reason we think that *substance "V"* and bradykinin are different, but closely related polypeptides.

Substance "V" is inactivated by different tissues, the most active being the kidney, followed by the spleen and the liver. The brain and red cells have nearly the same activity (fig. 4). This is also the sequence found for angiotensinase, the enzyme which destroys angiotensin. Since this enzyme is also irreversibly inactivated by incubation at pH 3.5 it is possible that both are the same peptidase.

When blood plasma is acidified at

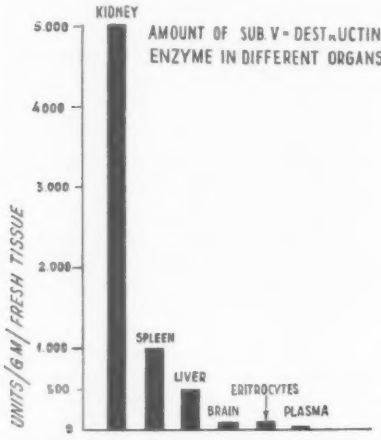

FIG. 4. — *The activity of every organ in expressed in Units per gram of fresh tissue. 1 Unit is the amount of enzyme which destroys 50 % of the vasodilator activity of 2 Units of substance "V" dissolved in 2 ml of saline at pH 7.4 after 30 minutes of incubation at 37° C.*

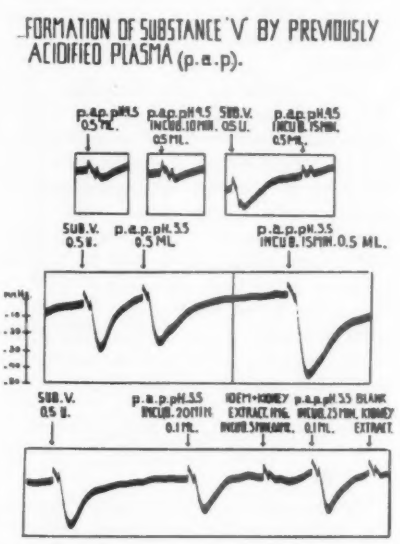

FIG. 5. — *Dog plasma was acidified at the pH chosen (4.5 or 3-5), incubated 30 minutes at 37° C and then brought to pH 7.4.* Above: *The incubation of previously acidified plasma to pH 4.5 does not give rise to a depressor substance.* Middle: *0.5 ml of plasma acidified at pH 3.5 gives rise to a depressor substance. The depressor action increases after 15 minutes incubation.* Below: *The depressor action is lost after 3 minutes incubation with 1 mg of fresh renal tissue.*

pH 3.5 and incubated at 37° C, a vasodilator substance is formed, which is destroyed rapidly by kidney extracts (fig. 5), suggesting its relation to *substance "V"*. The appearance of this substance in plasma, when the enzyme that destroys *substance "V"* is inactivated, brings in the question: is a *substance "V"* forming enzyme (plasmin ?) normally present in blood plasma, or its activation is due to the previous acidification?

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EL FACTOR HIPOFISARIO EN LA TUMORIGÉNESIS EXPERIMENTAL DEL OVARIO

E. Fels y V. G. Foglia
(Maternidad Ramón Sardá e Instituto de Fisiología de la Facultad de Medicina de Buenos Aires.)

Desde que se obtienen tumores experimentales en el ovario, es opinión unánime que la causa de la tumorigénesis reside en un desequilibrio hipófiso-ovárico provocado por la intervención (p. e., el injerto del ovario en el bazo o la ligadura del pedículo vascular del ovario). Se supone justificadamente que este desequilibrio consiste en una hiperfunción hipofisaria, la que estimula al ovario hasta que reacciona con la formación del tumor.

Tal opinión se basa en dos hechos: 1) en las modificaciones morfológicas que el ovario sufre en el curso del experimento, modificaciones que son idénticas a las que se observan cuando existe un aumento de las gonadotrofinas; 2) en la supresión de la formación del tumor por medio de las hormonas sexuales. Así Biskind y colab. (1) han observado que nunca se produce un tumor ovárico en el bazo en presencia de un ovario normal. Li y Gardner (2) impidieron la tumorigénesis en ratones inyectándoles estradiol o propionato de testosterona, encontrando lo mismo Lipschutz y colab. (3) en cobayas. Uno de nosotros (4) ha observado que las hormonas estrogénicas y androgénicas no sólo impiden la formación del tumor ovárico, sino que también provocan la regresión de un tumor ya formado. La explicación de estos fenómenos está dada por el efecto frenador sobre la hipófisis, característico de las hormonas sexuales. Es muy significativo que otra hormona sexual, la progesterona, no tiene acción alguna sobre el injerto ovárico intraesplénico (5), y no la tiene porque el efecto de esta hormona sobre la hipófisis es nulo o mínimo.

La tesis de la importancia fundamental de la hipófisis para la tumorigénesis experimental del ovario está, pues, bien apoyada por numerosas observaciones. Sin embargo, nadie ha tratado de demostrar en forma directa el papel que la hipófisis desempeña con respecto al desarrollo del tumor ovárico. El método inobjetable para realizar tal estudio es el método clásico para demostrar la función de una glándula, la extirpación de ella y la observación de las consecuencias de esta intervención.

A este respecto queremos mencionar que hace 16 años hemos realizado la hipofisectomía en ratas, sometidas, previa o posteriormente, a la ligadura del

pedículo vascular del ovario ([6]), con la intención de investigar el papel de la hipófisis en las modificaciones que experimenta el ovario ligado. Habíamos observado entonces solamente la formación de quistes, luteinización y atresia del ovario ligado; el descubrimiento de que la ligadura también es capaz de provocar tumores del ovario se hizo más tarde ([7]). Sin embargo, ya entonces se puso en evidencia que con la hipofisectomía se suprimen todas las modificaciones del ovario encontradas como consecuencia de la ligadura, experimentando el ovario ligado la misma atrofia que el ovario normal de la rata hipofisectomizada. Ésta fué la primera prueba directa de que la hipófisis es el órgano responsable de los procesos que conducen a la degeneración blastomatosa del ovario.

En el presente trabajo tratamos de demostrar el papel de la hipófisis en el desarrollo del injerto ovárico intraesplénico.

Sabemos que el ovario injertado en el bazo de una rata recién castrada "prende", con la formación ulterior de un tumor, en un 70 a 100 % de los casos ([8]).

Para estudiar el problema arriba expuesto hemos utilizado ratas hembras jóvenes de un peso inicial comprendido entre 115 y 130 gramos.

Se han formado distintos lotes para investigar la acción hipofisaria sobre la tumorigénesis en varios aspectos:

Lote I: En 12 ratas se efectúa hipofisectomía completa (comprobada más tarde por la necropsia) y 8 a 11 días después, la castración y autoinjerto de un ovario en el bazo; fueron sacrificadas de los 68 a los 94 días del injerto.

Se observó la reabsorción completa o atrofia macro y microscópica del injerto en todos los casos, o sea *regresión en el 100 %*.

Lote II: En 19 ratas se efectuó la hipofisectomía como en el grupo anterior. De los 14 a 21 días se realizó la castración e injerto intraesplénico de un ovario proveniente de una rata normal; el sacrificio tuvo lugar de 93 a 105 días después de la hipofisectomía.

El resultado fué reabsorción o atrofia del injerto en todos los casos, es decir *regresión en el 100 %*.

Lote III: 25 ratas normales castradas a las que se injerta el ovario de una rata hipofisectomizada 8 a 22 días antes; sacrificadas entre 68 y 121 días después de la implantación.

Resultado: reabsorción o atrofia del injerto en 21 casos. *Regresión en el 84 %*.

Lote IV: 9 ratas con un tumor del injerto ya formado y verificado por laparatomía y biopsia del tumor. Hipofisectomía, sacrificadas entre 23 y 59 días de ésta. Disminución y atrofia macro y microscópica de mayor o menor grado en todos los casos. (Ver fig. 1 y 2). *Rgresión en el 100 %*.

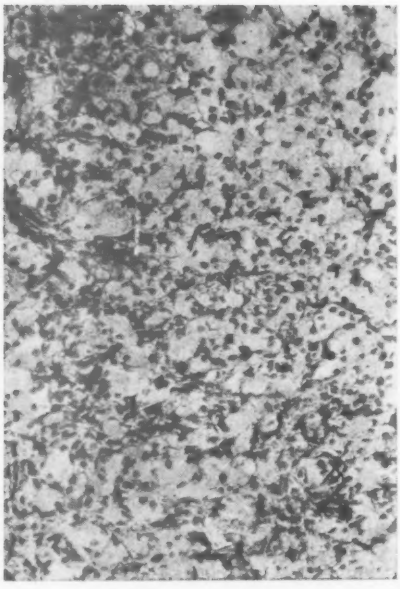
Lote V (control): 19 ratas; a los 150-212 días de la castración con autoinjerto del ovario en el bazo, se ha encontrado una proliferación blastomatosa del injerto en 14 ratas (70 %) y atrofia o reabsorción en 5 (26 %).

Estos últimos resultados están de acuerdo con los de Kullander ([9]) quien encontró que la hipofisectomía en una rata portadora de injerto blastomatoso del ovario es seguida por modificaciones regresivas de éste.

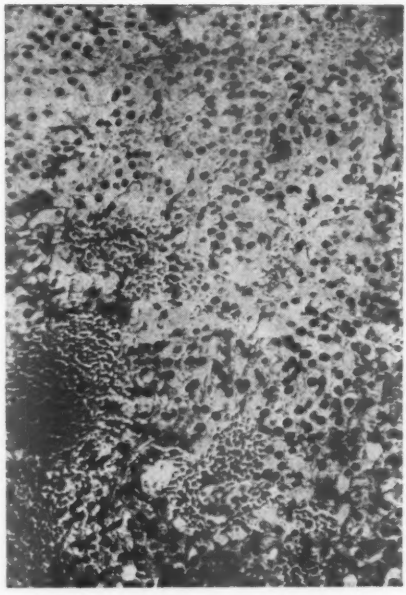
Resumen

Un ovario injertado en el bazo de una rata hipofisectomizada y recién castrada, siempre se atrofia.

Cuando se injerta el ovario de una rata hipofisectomizada, éste "prende" sólo en un 16 % (el porcentaje correspondiente al ovario de rata normal es de 70 a 100 %). La hipofisectomía en ratas con un tumor ovárico intraesplénico ya desarrollado, produce la regresión del tumor.



1. — *24-VI-58: Castración autoinjerto. 15-I-59: Biopsia: tumor de 1 cm aproximadamente; imagen de luteoma. Hematoxilina - eosina.*



2. — *23-III-59: Hipofisectomía. 21-VI-59: Tumor de 3 mm aproximadamente, color amarillo pardo; necrobiosis. Hematoxilina - eosina.*

Imágenes observadas en la misma rata (lote IV, rata 79) en las fechas señaladas en las respectivas leyendas.

Conclusión

Estos resultados comprueban en forma directa que la hipófisis es el órgano responsable de la tumorigénesis experimental del ovario.

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HYPOTHALAMIC BLOOD PRESSURE RESPONSES IN HYPOTHERMIC RATS

EDUARDO MOACYR KRIEGER
(Department of Physiology — Medical College of Ribeirão Prêto — Ribeirão Prêto, São Paulo, Brasil.)

IT HAS been shown that progressive cooling depresses the pressor reflexes from the carotid pressoreceptors [1-5] and suppresses them below a certain degree of hypothermia. This depressant action of cooling was attributed to a central effect of cold upon the autonomic nervous centers. Therefore, to explain the maintenance of the arterial pressure in the cooled animals at low hypothermic levels the activity of vasomotor centers located in the spinal cord was suggested [5] and/or an increase in blood viscosity [6,7]. However, there was no direct evidence that progressive cooling has, at all levels of hypothermia, a depressive action on the autonomic centers which control the blood pressure.

By stimulating the posterior hypothalamic centers in cooled cats Koella, Ballin and Gellhorn [8] found a decrease in the pressure responses below 30°C and concluded that hypothermia below that temperature produces a hypoexcitability of these centers. However, the authors also signaled a marked diminution on the pressor effect of epinephrine during hypothermia, indicating a profound alteration on the sensitivity of the effectors to the sympathetic mediators.

More recently, it was emphasized that cooling produces in different parts of the nervous system a phase of hyperresponsiveness between temperatures of 35°C to 25°C [9] before the classical depressant action. Therefore, it was thought worthwhile to study in hypothermic rats the evolution of the blood pressure responses to hypothalamic stimulation and, at the same time, the alteration produced by hypothermia on the pressor effects of epinephrine and levarterenol.

MATERIAL AND METHODS

Adult male Wistar rats anesthetized with pentobarbital sodium intraperitoneally (4 mg/100 g) were used through out the study. Mean arterial blood pressure was recorded with a mercury manometer by direct cannulation of the left carotid artery with polyethylene tubing (0,95 mm). Blood clotting was avoided by the injection of 0,2 ml heparin (Abbot-1000U/ml) intravenously. The intravenous injections were made through a polyethylene tube inserted

into the external jugular vein, in volumes no larger than 0,3 ml. Heart rate and electrocardiograms were recorded by needle electrodes (lead II) by an electroencephalograph with a speed of 16-32 mm/sec. The rats were cooled by placing them in a metal tambour (23 cm length, 9 cm diameter) with double wall through which cool water circulated from a reservoir moved by a pump. This method provided a progressive cooling, quite reproductible in the different animals. Colonic temperatures were measured by a mercury thermometer inserted into the recto-sigmoid. No additional anesthetic was administered to the rats during the cooling time. Artificial respiration was provided by a pump with a stroke volume adjusted individually to a rate of 40/min. Bilateral adrenalectomy was performed in a one stage operation by a dorso-lateral approach.

The posterior hypothalamus was stimulated through steel bipolar coaxial electrodes (0,475 mm diameter) insulated to the tip and implanted according to the stereotaxic technique with a Krieg-Johnson apparatus and with the coordinates described by Krieg (10). The position of the electrode in the posterior hypothalamus was checked in several preparations by macroscopic and microscopic examinations. The electrical stimuli were square waves of 2 msec duration, 40/sec, variable voltage, delivered by a Grass Model S_4-C stimulator through an isolation unit.

Epinephrine (chlorhydrate-Parke Davis) and levarterenol (bitartrate-Byk) were diluted with a preservative solution (1 g chlorobutanol and 1 g sodium hyposulphite in 1000 ml H_2O). Hexamethonium chloride was used as ganglionic blocking agent (1-3 mg/rat).

Results

In order to estimate the method of cooling employed, in a preliminary series of 9 rats the modifications produced by hypothermia on the colonic temperature, arterial blood pressure and heart rate were observed. Figure 1 summarizes the mean values obtained every 10 minutes during the observation time. The heart rate decreased with colonic temperature in an apparently linear fashion. These data and the alterations observed in the electrocardiograms agreed closely with the results first described in hypothermic rats by Hamilton et al. (11). Blood pressure fell slightly until 20°C, below this temperature, however, the decrease became more marked, but in a less pronounced way than that described by Crismon (12)

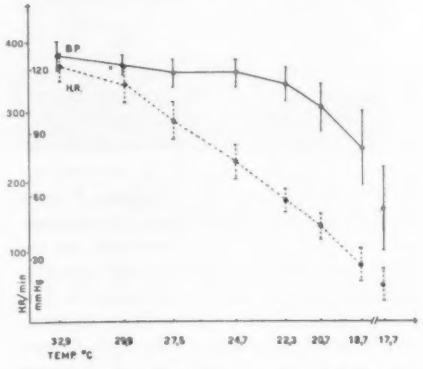

FIG. 1. — *Average blood pressure and heart rate with its fiducial limits of 9 rats submitted to hypothermia. Observations made every 10 minutes. Values at 17,7°C belong to only 7 of the 9 animals.*

and Hansen (13) in hypothermic rats anesthetized with pentobarbital sodium. The evolution of the blood pressure was similar to that described by Bullard (6) in rats with ether anesthesia, and by Chevillard et al. (14) in rats cooled by Giaja's method. The presence of shivering and the maintenance of the arterial blood pressure indicated that the anesthetic used did not produce a great depression on the animals submitted to hypothermia.

1 — The effects of hypothalamic stimulation on blood pressure and heart rate, at normal temperature.

The stimulation of the posterior hypothalamus in 9 rats mantained at normal temperature determined only rises in pressure which were proportional to the intensity of the stimuli used until the maximal responses were reached (usually higher than 100 mm Hg). In three animals the possibility to obtain hypotensive effects was investigated by stimulating different zones of the hypothalamus with stimuli of varying parameters. Except some hypotensive responses which disappeared by the instauration of artificial respiration a clear fall in pressure produced by hypothalamic stimulation was not observed. The secondary rises in pressure determined by hypothalamic stimulation as described by Scherrer and Friedman ([15]) and attributed to a vasopressin liberation were not observed. Ganglionic blockade with hexamethonium completely abolished the pressor rises. Exceptionally strong stimuli produced an increase in the heart rate not larger than 10 % of the control values. The latency of the pressor responses were approximately one second, similar to that described by Karplus and Kreid ([16]) for the cat. The peaks of the pressor rises were reached in 4-6 sec, therefore 10 sec was the total duration established for the hypothalamic stimulation.

In order to assess the stability of the preparation, five rats were maintained up to three hours and a standard stimulus repeated every fifteen minutes. Table I shows the results obtained in the first two hours of observation. With the lowering of the blood pressure the pressor responses became higher, confirming the observations made by Scherrer and Friedman ([15]). No signals of deterioration were observed; even at the end of the third hour proportionality between the intensity of stimulus and the response was still present.

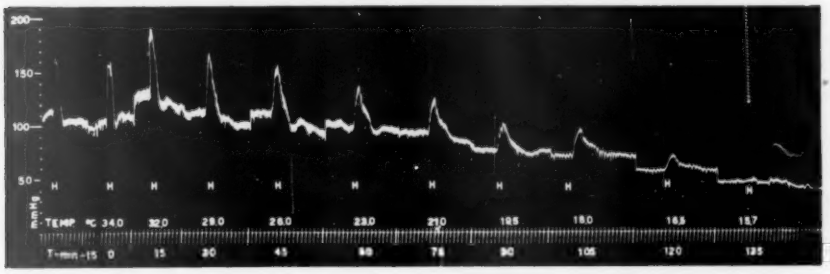

FIG. 2. — *Hypothalamic blod pressure responses in a rat (300 g) submitted to hypothermia. H=monophasic pulses, 40/sec, 2 msec, 2,5 V during 10 sec. At 15°C stimuli of 30 V produced no responses. Time: 10 sec.*

2 — Hypothalamic stimulation in hypothermic rats.

After the relation between intensity of stimuli and of pressor responses had been observed, a standard voltage (2-5V) which gave an increase in pressure of 40-50 mm Hg was individually established in 7 rats. This stimulus was usually repeated 15 minutes after, immediately before the beginning of cooling. All the cooled animals reacted in the same direction. Table II shows that

TABLE I

Presure responses to hypothalamic stimulation. Five rats stimulated every 15 minutes (Mean ± S.E.).

T. min.	Blood pressure mm Hg	Blood pressure responses mm Hg
0	106 ± 6	33 ± 6
15	110 ± 6	30 ± 3
30	91 ± 9	41 ± 7
45	95 ± 9	40 ± 4
60	94 ± 7	45 ± 8
75	88 ± 9	48 ± 7
90	89 ± 9	43 ± 6
105	89 ± 8	42 ± 6
120	84 ± 11	43 ± 10

3 — Epinephrine and levarterenol during hypothermia.

Figure III shows the effects of hypothermia on the pressor activities of epinephrine and levarterenol (0,3 micrograms) obtained in 12 rats, the injections being repeated every 15 minutes during the cooling time. Levarterenol presented a reduction of approximately 50 % on its pressor effects at all levels of hypothermia studied (until 19°C in this series). On the pressor activities of epinephrine hypothermia determined a 50 % reduction until about 30°C. Thereafter progressive improvement of the pressor responses below that

TABLE II

Hypothalamic stimulation in 6 rats submitted to hypothermia. Mean±S.E. of the first 90 minutes.

T. min.	Temp. °C	Heart rate beat/min.	Blood pressure mm Hg	Bl. pressure response mm Hg	Bl. pressure response duration (sec.)
0	33,4 ± 0,3	352 ± 11	111 ± 3	48 ± 4	17 ± 2
15	31,4 ± 0,4	323 ± 14	116 ± 8	45 ± 3	21 ± 2
30	28,3 ± 0,4	257 ± 11	95 ± 10	48 ± 6	32 ± 4
45	25,6 ± 0,4	202 ± 13	96 ± 9	37 ± 4	45 ± 4
60	23,1 ± 0,5	161 ± 12	91 ± 10	37 ± 5	64 ± 9
75	21,4 ± 0,4	119 ± 13	83 ± 7	33 ± 5	82 ± 12
90	19,9 ± 0,3	86 ± 9	74 ± 5	29 ± 6	86 ± 11

until the colonic temperature of 28°C was reached, the hypothalamic pressor responses were almost unchanged; at 26°C they were diminished and below that temperature the decrease became progressively more pronounced until no more responses were obtained, even with stimuli as high as 20-30 V at 19-14°C, as illustrated in figure II. The latency of the responses increased from 1 second at normal temperature to 4-5 sec at 20°C, and 8-9 sec at 14-15°C. No significant modifications in the heart rate were observed during the hypothalamic stimulation.

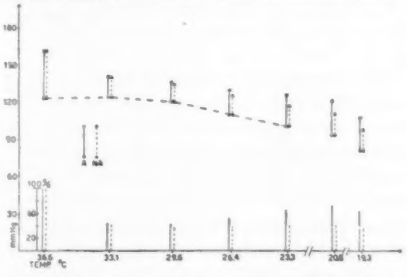

FIG. 3. — *Pressor effects of 0,3 micrograms of epinephrine (A) and levarterenol (NA) in 12 rats submitted to hypothermia. Values at 20,8°C and 19,3°C belong to only 10 and 7 rats, respectively. Injections repeated every 15 minutes during the cooling time.*

temperature was observed until the maximal recuperation was reached at 20°C when epinephrine exhibited about 75% of its control value. Below the colonic temperature of 20°C the responses decreased again. The duration of the catecholamines pressor effects decreased until 26°C; however, below that temperature they became gradually larger, being twice as long at 20° than at normal temperature, as illustrated in figure IV. The onset of the effect as well as the time required for the development of the peak responses was similarly prolonged during hypothermia.

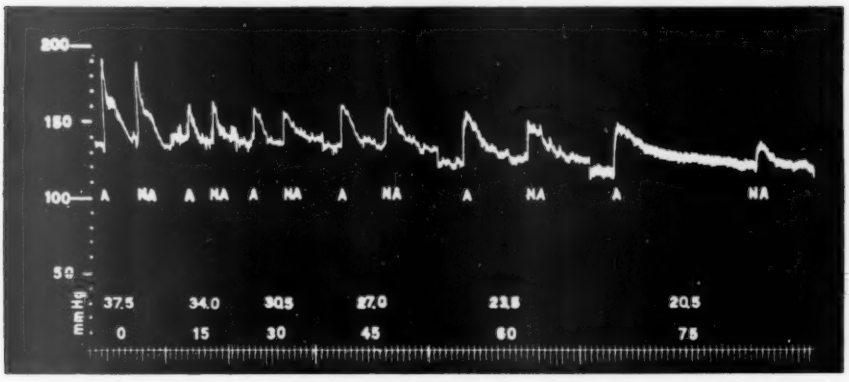

FIG. 4. — *Effects of cooling on the pressor activities of 0,3 micrograms epinephrine (A) and levarterenol (NA). Time: 10 sec.*

4 — Hypothalamus and epinephrine during hypothermia.

The experimental conditions were not the same in the 2 groups of rats in which the effects of cooling on the pressor activities of the catecholamines and on the hypothalamic pressor responses were studied. Therefore, in another series of 9 rats the pressor test were repeated in the same preparation. The averaged data of 7 out of 9 rats of this group are found in figure V, the 2 animals whose data are not tabulated exhibited results in the same direction but were observed only until a colonic temperature of 20°C was reached.

The alterations produced by hypothermia on the pressor responses were similar to those previously described. The depression on the hypothalamic effects occurred at about 27°C but only below 25°C the reduction became more pronounced than the one simultaneously induced by the pressor effects of epinephrine. At all the hypothermic levels studied above 25°C the pressor effects produced by hypothalamic sti-

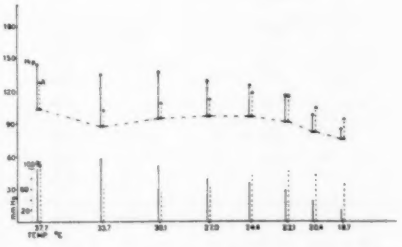

FIG. 5. — *Effects of hypothermia on the blood pressure responses to hypothalamic stimulations (Hip) and 0,3 micrograms of epinephrine (A) in 7 out of 9 cooled rats. Pressor tests repeated every 15 minutes during cooling time.*

mulation were proportionately much more effective than those determined by epinephrine injections.

5 — Effect of acute adrenalectomy on the hypothalamic pressor responses.

The pressor action of epinephrine at low colonic temperatures has been found to be more potent than that of levarterenol. Therefore, it was thought worthwhile to investigate further which of the two substances more precisely indicates the alterations produced by hypothermia on the blood pressure responsiveness to the mediator(s) liberated by hypothalamic stimulation. Since in a discharge of the sympathetic-adrenal system the only important source of epinephrine is the adrenal gland [17, 18] the effect produced by removal of the adrenal glands on the hypothalamic pressor responses was studied. Table III shows the rises in pressure due to hypothalamic stimulation obtained 15 minutes before, immediately before and 15 minutes after bilateral adrenalectomy. Extirpation of the adrenal glands did not produce any significant alterations on the blood pressure responses elicited by the hypothalamic stimulation. The control blood pressure

TABLE III

Effect of acute adrenalectomy on the hypothalamic pressure responses, 15 minutes before (−15) immediately before (0) and 15 minutes after (+15) bilateral adrenalectomy. In parentheses, the control blood pressures.

RAT	Stimuli Volts	Blood pressure responses (mm Hg)					
		− 15 min.		0		+ 15 min.	
1	2,0	(143)	20	(133)	20	(133)	20
	2,5		40		48		52
	3,0		58		70		64
	3,5		60		75		75
	4,0		71		80		82
2	2,5	(113)	14	(106)	18	(102)	24
	3,0		32		34		40
	3,5		43		40		42
	4,0		56		57		52
3	2,5	(112)	14	(98)	22	(84)	18
	3,0		28		32		33
	3,5		45		53		52
	4,0		56		56		55
4	5,5	(114)	32	(100)	28	(86)	27
	6,0		52		44		46
	6,5		61		58		54
5	4,8	(104)	38	(99)	36	(84)	30
	5,3		60		62		62
	5,8		72		71		71
6	2,0	(110)	26	(102)	20	(106)	20
	2,5		47		48		46
	3,0		64		60		51

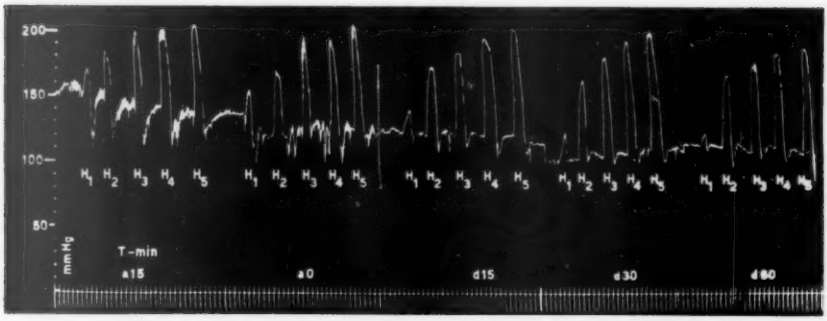

FIG. 6. — *Effect of the extirpation of the adrenal glands on the hypothalamic pressor responses. Monophasic pulses 40 sec. 2 msec, during 10 sec.* H_1*=2 V,* H_2*=2,5 V,* H_3*=3 V,* H_4*=3,5 V and* H_5*=4 V,* a_{15} *and* a_0*. 15 minutes and immediately before bilateral adrenalectomy.* d_{15}*,* d_{30} *and* d_{06}*. 15, 30 and 60 min after adrenalectomy. Time: 10 sec.*

remained almost constant in spite of the surgical trauma. Figure VI illustrates one of the experiments.

6 — Hypothalamus and levarterenol in hypothermic adrenalectomized rats.

The evidences that the catecholamines of the adrenal glands were not important to the blood pressure responses produced by hypothalamic stimulation, at normal temperature, may be not true during hypothermia where the physiologic conditions are quite different. To avoid this possibility the effects of cooling on the hypothalamic pressure responses were compared with those simultaneously determined in a equipotent pressor responses due to levarterenol injections, in 8 rats acutely adrenalectomized.

Table IV shows that the pressor effects of hypothalamic stimulation during hypothermia were much more effective than those produced by levarterenol until the temperature of 20°C was reached. Only below 20°C the increases in blood pressure produced by the hypo-

TABLE IV

Blood pressure responses to hypothalamic stimulation and levarterenol (0,6-0,9 micrograms) in 8 rats acutely adrenalectomized and submitted to hypothermia.

T min.	Temp. °C	Heart rate beat/min.	Pressure mm Hg	Blood pressure responses			
				Hypothalamus Stimulation		Levarterenol	
				mm Hg	duration (sec.	mm Hg	duration (sec.)
0	36,1 ± 0,1	372 ± 11	96 ± 5	48 ± 2	18 ± 1	47 ± 2	84 ± 6
15	32,1 ± 0,2	350 ± 5	92 ± 7	43 ± 2	22 ± 1	27 ± 2	69 ± 5
30	28,4 ± 0,2	277 ± 6	88 ± 5	37 ± 3	29 ± 3	20 ± 1	62 ± 4
45	25,1 ± 0,2	218 ± 9	81 ± 3	31 ± 3	36 ± 3	19 ± 2	77 ± 6
60	22,2 ± 0,3	154 ± 3	73 ± 3	23 ± 4	44 ± 5	19 ± 1	107 ± 4
75	19,6 ± 0,2	109 ± 4	59 ± 4	14 ± 4	37 ± 6	16 ± 2	142 ± 7
90	17,6 ± 0,2	81 ± 3	48 ± 5	8 ± 3	31 ± 6	15 ± 1	164 ± 12

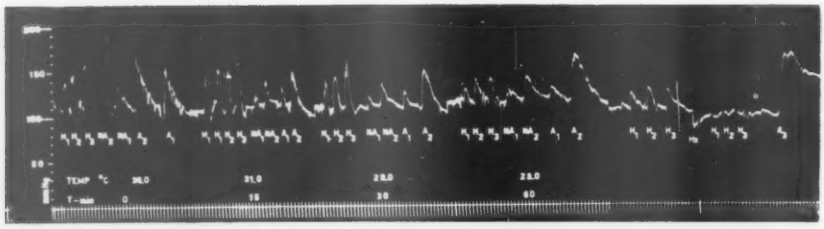

FIG. 7. — *Blood pressure responses to hypothalamic stimulation (H) epinephrine (A) and levarterenol (NA) in a rat (300g) submitted to hypothermia H_1, H_2 and H_3=4,5 and 6 V A_1, A_2 and A_3 = 0,3, 0,9 and 1,8 micrograms of epinephrine NA_1 and NA_2=0,3 and 0,9 micrograms of levarterenol. H_x=.1 mg hexamethonium chloride. Time: 10 sec.*

thalamic stimulation became smaller than those determined by levarterenol injection.

Finally, in 4 rats cooled with intact adrenal glands the effects of hypothermia on several pressor tests instead of the standard one as used before, were studied. Figure VII shows that the results obtained in one of the rats, representative of what has occurred with the animals of this group, were essentially the same of those previously described. In this series hexamethonium chloride (1-3 mg) was injected at low colonic temperatures and completely abolished the rises in pressor responses produced by hypothalamic stimulation.

DISCUSSION

In hypothermic rats the hypothalamic pressor responses remained normal, or slightly increased, until colonic temperatures near 28°C were reached. At the same time a reduction of approximately 50 % in the pressor activities of epinephrine and levarterenol was observed.

If it is assumed that hypothermia determines an initial phase of enhancement of the hypothalamic responses, the discrepancy in the cooling effects upon the pressor increases due to hypothalamic stimulation and on those obtained by the sympathetic mediators, could be explained. It is worth to note that this initial hyperresponsiveness has been shown for other structures in the central nervous system as spinal cord, cerebral and cerebellar cortex (9, 19, 20). When both pressor responses, one due to hypothalamic stimulation and the other to levarterenol, are simultaneously studied, it is possible to observe a larger depressive action of cooling on the hypothalamic responses only below 20°C.

A facilitatory action elicited by hypothermia at the spinal cord level should, at least partially, explain the increased number of sympathetic neurons activated by electrical stimuli on the posterior hypothalamus. No potentiations have been mentioned when preganglionic fibers of the sympathetic system were activated during hypothermia [21-23]. The effects of cooling on the hypothalamic centers themselves can not be assessed directly by our results: they suggest, however, that a depressant action determined by cooling can only be assumed, with more probability below a colonic temperature of 20°C.

The observed actions of cooling on the vasoconstrictor effects of epinephrine and levarterenol [24, 25] may explain the decreased blood pressor responsiveness exhibited by these two catecholamines during hypothermia. The effects of epinephrine on the myocardial con-

tractility has been found almost normal during hypothermia ([26, 27]), but levarterenol when present, diminished this effect ([28]). This discrepancy on the cardiac effects elicited by epinephrine and levarterenol should explain, at least partially, the higher pressor activity manifested by epinephrine at low colonic temperatures.

The pressor effect of a large dose of epinephrine was found normal in cooled chickens and turtles ([29, 30]). The blood responses to epinephrine has been referred to be increased in rats ([14]) and inverted in cats ([8]) submitted to hypothermia. Some of the discrepancies between these results and those here reported may be due to differences in the animal species and methods of cooling used. The modifications produced by hypothermia on the pressor effects of epinephrine and levarterenol presently observed are similar to those described in hypothermic dogs at body temperatures of 22-25°C ([28]).

It seems that the heart rate of the rat is, normally and during hypothermic state, under near the maximal influence of the sympathetic tonus; therefore, epinephrine injections, even with vagus nerve blockade, does not produce an appreciable tachycardia ([31, 32]). This phenomenon may explain the absence of heart rate increases when the posterior hypothalamus was stimulated at normal or low colonic temperature.

Although there are evidences that the hypothalamic stimulation liberate abundant pressor amines from the adrenal medulla, as shown by Houssay and Molinelli ([33]) and confirmed by others ([34-37]), the relative contribution of this secretion to the pressor effects has not been precisely established. Karplus and Kreidl observed that the hypothalamic pressor responses can be obtained in adrenalectomized cats ([16]). Magoun et al. ([38]) also showed that the pressor effects elicited by the hypothalamic stimulation in cats were due mostly to an activation of the sympathetic system, with no participation of the adrenal medullary secretion. Our results also indicate that in rats when hypothalamic areas are stimulated, the observed pressor effects remain inalterable with extirpation of the adrenal glands.

These experiments are compatible with the idea that the autonomic nervous centers may exert their influences upon the blood pressure until quite low body temperature (near 20°C) are reached. When interpreting the depressive effect of cooling on pressor reflexes, the great alteration exhibited by the blood pressure responsiveness to the sympathetic mediator(s) must necessarily be considered.

Summary

In hypothermic rats the pressor responses elicited by stimulation of the hypothalamus remained unchanged, or slightly increased, until 28°C. Thereafter, the responses became progressively smaller until they disappeared at 19-14°C. At all studied levels of hypothermia, the blood pressure responses to levarterenol were approximately one-half of the control values. Epinephrine exhibited the same reduction until near 26°C, below that temperature its pressor effects increased, attained maximal recuperation at 20°C (70-90 % of the control values) and thereafter the responses declined again. Although a real diminution of the hypothalamic pressor effects occured below 28°C, when the decrease of the blood pressure responsiveness to epinephrine or to levarterenol was considered, the depression produced by hypothermia on the hypothalamic responses became apparent only below 25 or 20°C, respectively.

Hypothalamic stimulation, as well as injection of pressor amines, produced no increases in the heart rate at any le-

vel of body temperature; exceptionally strong stimuli determined a discrete tachycardia.

Bilateral adrenalectomy produced no significant changes in the intensity and duration of the hypothalamic blood pressure responses.

We wish to acknowledge revision of the manuscript by Dr. Miguel R. Covian.

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RECIENTES PROGRESOS EN EL CONOCIMIENTO DEL METABOLISMO DEL GLUCÓGENO

LUIS F. LELOIR Y CARLOS E. CARDINI
(Instituto de Investigaciones Bioquímicas "Fundación Campomar" y Facultad de Ciencias Exactas y Naturales, Obligado 2490, Bs. Aires, Argentina.)

HACE poco más de 100 años que Claude Bernard (1857), después de haber demostrado la función glucogénica del hígado anunció el aislamiento de la "substance glycogenique animale". Con su habitual precisión Bernard describió las propiedades de esta substancia de reserva que había obtenido del hígado, a saber: la solubilidad en el agua, la resistencia al álcali, el color rojo que da con iodo y la hidrólisis con ácido que libera azúcar reductor.

Durante estos 100 años el glucógeno ha sido estudiado por los más hábiles investigadores. La estructura química recién pudo estudiarse con más detalle con los métodos de metilación que permitieron establecer los principales tipos de unión entre las unidades de glucosa. Al mismo tiempo, los estudios puramente químicos se fueron complementando y extendiendo con los datos aportados por los métodos físicos, físicoquímicos y por la degradación y síntesis enzimática.

Abreviaturas usadas:

UDPG: uridina difosfato glucosa; UDP: uridina difosfato; UTP: uridina trifosfato; ATP: adenosina trifosfato; PP: pirofosfato inorgánico; P_i: fosfato inorgánico.

Desde el punto de vista fisiológico y gracias a técnicas analíticas simples, se obtuvo un panorama bastante completo del metabolismo del glucógeno, aún cuando los procesos enzimáticos comenzaron a conocerse recién en 1935. Hasta ese año era generalmente aceptado que la degradación del glucógeno en los tejidos animales se iniciaba por una hidrólisis enzimática dando glucosa o tal vez una "forma especialmente reactiva de la hexosa". Fué en el año 1936 que Parnas y Baranowski descubrieron que en extractos de músculo de conejo se formaba a partir de glucógeno y fosfato, un éster fosfórico estable de glucosa: el éster de Embden (mezcla de glucosa-6-fosfato y fructosa-6-fosfato) (Ostern y colaboradores, 1936). Parnas (1937) bautizó esta reacción con el nombre de "fosforólisis" y a la enzima que lo cataliza: "fosforilasa".

El descubrimiento de este proceso fundamental puede remontarse a los clásicos trabajos de los esposos Cori (Cori y Cori, 1932; Hegnauer y Cori, 1934; Cori y colaboradores, 1937) con músculos aislados estimulados eléctricamente o tratados con adrenalina. En esas condiciones el glucógeno y el fos-

fato inorgánico desaparecen y aparecen hexosa-fosfatos. Cori y Cori (1936) con extractos lavados de músculo de sapo e incubados corto tiempo con fosfato y cantidades catalíticas de ácido adenílico, demostraron que había una discrepancia entre la cantidad de fosfato inorgánico que desaparecía y la de éster fosfórico reductor que se formaba. Estas discrepancias desaparecían en incubaciones más prolongadas y de allí se dedujo que debía existir una substancia intermedia entre el glucógeno y el hexosa–6–fosfato. Esta substancia resultó ser un éster fosfórico lábil de la glucosa, el glucosa-1-fosfato llamado también éster de Cori (Cori y colaboradores, 1937-38).

La formación de glucógeno a partir del glucosa-1-fosfato fué anunciada en el año 1939 por Kiessling usando una enzima de levadura y por Cori y col., en el mismo año con enzima de músculo. Este hallazgo fué importante puesto que por primera vez se había logrado sintetizar "in vitro" un polímero natural. También se logró aislar fosforilasa de las plantas y con ella se pudo sintetizar almidón (Hanes, 1940).

A consecuencia de estos descubrimientos se creyó que enzimas similares a la fosforilasa debían ser responsables de muchas otras reacciones sintéticas. Efectivamente en 1943 (Doudoroff y colaboradores) se halló en microbios (Pseudomonas) una enzima que cataliza la reacción reversible:

$$\text{Sacarosa} + \text{fosfato inorgánico} \rightleftharpoons \text{glucosa-1-fosfato} + \text{fructosa}$$

A raíz de este hallazgo se consideró probable que la síntesis de sacarosa en las plantas debía efectuarse de la misma manera. Sin embargo la enzima correspondiente no pudo hallarse en las plantas y el equilibrio de la reacción tiende más bien a la degradación que a la síntesis de sacarosa. Más recientemente se han encontrado enzimas que catalizan la fosforólisis de la maltosa (Fitting y Doudoroff, 1952) y de la celobiosa (Sih y colaboradores, 1957). La opinión general actual es que estas fosforilasas funcionan en las células en el sentido de la degradación y no de la síntesis.

Un mecanismo de síntesis algo diferente fué descubierto en 1955 (Leloir y Cabib). Es el de la trehalosa fosfato de acuerdo a la siguiente ecuación:

$$\text{UDPG} + \text{glucosa-6-fosfato} \rightarrow \text{trehalosa fosfato} + \text{UDP}$$

Esta reacción se diferencia de las anteriores en que el dador de glucosa no es el glucosa-1-fosfato sino el UDPG.

Seguidamente (Cardini y colaboradores, 1955) fué demostrado en plantas un proceso similar para la síntesis de la sacarosa.

$$\text{UDPG} + \text{fructosa} \rightarrow \text{sacarosa} + \text{UDP}$$

y de sacarosa fosfato (Leloir y Cardini, 1955)

$$\text{UDPG} + \text{fructosa-6-fosfato} \rightarrow \text{sacarosa fosfato} + \text{UDP}$$

Se han estudiado otras reacciones de transferencia similares que dan lugar a la síntesis de glucurónidos, glucósidos, lactosa, celulosa y quitina.

Recién en 1957, cien años después del descubrimiento del glucógeno se halló una enzima en el hígado que transfiere glucosa del UDPG al glucógeno y que parece ser responsable de la síntesis "in vivo" (Leloir y Cardini, 1957).

Estructura del glucógeno

Los distintos tipos de glucógeno aislados de tejidos por los métodos clásicos son todos macromoléculas altamente ramificadas, compuestas por varios miles de cadenas. La determinación del peso molecular muestra que los glucógenos están entre los más grandes polímeros naturales. Todas las muestras analizadas parecen ser polidispersas y en general se acepta que sus pesos moleculares

son del orden de uno a diez millones (Greenwood, 1952; Manners, 1957).

Las cadenas formadas por uniones $\alpha\ 1 \rightarrow 4$ tienen en término medio 10 a 14 unidades de longitud. Estas cadenas a su vez están combinadas entre sí por uniones $\alpha\ 1 \rightarrow 6$ formando las ramificaciones. Las cadenas individuales varían considerablemente en longitud. Un glucógeno de peso molecular de 5 millones contiene unos 31.000 residuos de D-glucosa y alrededor de 2500 uniones $\alpha\ 1 \rightarrow 6$ y el mismo número de grupos finales correspondientes a cadenas exteriores de 7 a 13 unidades de glucosas de largo. En cambio el número de unidades entre cada punto de ramificación oscila entre 3 y 5 unidades. Esto da por resultado una molécula sumamente compacta. La forma total no parece ser esférica, sino más bien asimétrica siendo la relación axial de 1.8/2.8 para el glucógeno del hígado de conejo, y 1/11 para el del *M. tuberculosus*.

En lo que respecta al estado y peso molecular del glucógeno dentro de la célula hay todavía algunos aspectos en controversia. En 1942 Lazarow obtuvo a partir de papilla de hígado una fracción que denominó "glucógeno particulado". Esta fracción sedimenta cuando se centrifuga a velocidad mediana (14,000 r.p.m.) junto con los microsomas, formando una pastilla semitransparente que se adhiere fuertemente al fondo de los tubos. Este precipitado está constituído por glucógeno casi puro y por métodos apropiados se puede purificar hasta que el contenido en proteínas sea menor que el 0.005 %. El peso molecular es muy elevado siendo del orden de 2×10^8.

Stetten y Stetten (1957) también han obtenido preparaciones de muy elevado peso molecular por extracción con ácido tricloroacético en frío. El peso molecular del glucógeno obtenido por el clásico método de calentar con álcali es siempre menor que el de los antes mencionados y por lo tanto existe siempre el problema de cuál de los dos tipos representa mejor el estado real del glucógeno en la célula. Puede ser que el obtenido con álcali sea un producto de degradación o que el glucógeno "particulado" o el obtenido con tricloroacético sean agregados de varias moléculas más pequeñas unidas por impurezas. Como ejemplo de esto último se puede mencionar la acción de algunas proteínas, en particular la concanavalina, que producen agregación de las soluciones de glucógeno.

Biosíntesis del glucógeno

Cuando se logró sintetizar glucógeno "in vitro" con fosforilasa y glucosa-1-fosfato se concluyó inmediatamente que la síntesis "in vivo" tenía lugar por el mismo mecanismo. Había sin embargo algunos puntos difíciles de explicar. Uno de ellos se refiere al equilibrio de la reacción. Esta reacción puede escribirse en la siguiente forma:

Glucosa-1-fosfato + aceptor $\rightleftharpoons$ glucosil $\alpha\ (1 \rightarrow 4)$ aceptor + fosfato inorgánico

El equilibrio depende de la relación (fosfato inorgánico) / (glucosa-1-fosfato) y del pH. Así a pH 7, cuando la relación P_i/G-1-P tiene un valor de 3.2 la reacción está en equilibrio. Cuando la relación sobrepasa ese valor el glucógeno se degrada y a la inversa se sintetiza con valores más bajos. Ahora bien, en los tejidos la relación P_i/G-1-P es casi siempre mucho mayor que 3.2 y por lo tanto la fosforilasa debería actuar en el sentido de la degradación. Esta objeción fué dejada de lado aduciendo que las concentraciones en el sitio de síntesis podrían ser distintas que las obtenidas por dosaje del tejido entero, lo cual puede ser cierto.

Otros resultados que hicieron dudar sobre la intervención de la fosforilasa en la síntesis del glucógeno "in vivo" surgieron a raíz de experimentos sobre acción de la adrenalina. Esta substan-

cia produce glucogenolisis y Cori y Sutherland (cf. Sutherland, 1951), observaron que produce también un aumento en la actividad de la fosforilasa. Por lo tanto si al aumentar la fosforilasa se produce mayor glucogenolisis resulta difícil comprender cómo puede esta enzima determinar la síntesis del glucógeno.

Resultados similares a los obtenidos con la adrenalina fueron obtenidos con glucagon o sea que también con esta substancia se produce glucogenolisis y aumento de actividad de la fosforilasa.

Experimentos coincidentes se han llevado a cabo con papilla de músculo. Así Robbins y colaboradores (1959) agregando glucosa marcada obtuvieron una pequeña incorporación de la marca en el glucógeno. Si además agregaban fosforilasa-kinasa para aumentar la cantidad de fosforilasa activa, la incorporación en el glucógeno se hacía menor.

También merecen ser mencionados los experimentos de Niemeyer y colaboradores (cf. Niemeyer, 1955) quienes usando cortes de hígado llegaron a la conclusión de que existe un mecanismo de síntesis de glucógeno distinto del conocido hasta entonces.

El medio iónico también interviene en la regulación del contenido en glucógeno. Así, si se incuban cortes de hígado en líquidos con variadas relaciones de Na^+ y K^+ se observa que el glucógeno aumenta en los medios con alta concentración de K^+ y baja cuando la concentración del Na^+ es alta. También en estos casos se ha encontrado que a mayor glucogenolisis corresponde mayor actividad de la fosforilasa. Evidentemente estos hechos son sugestivos pero no prueban en qué dirección actúa la fosforilasa "in vivo". Tal vez son más probatorias las observaciones que se relatan más adelante sobre enfermos que tienen glucógeno en sus músculos pero no fosforilasa (Mommaerts y colaboradores, 1959; Schmid y colaboradores, 1959).

Las sospechas de que pudiera existir un mecanismo de síntesis del glucógeno diferente del que parte directamente del glucosa-1-fosfato, se vieron confirmadas con el hallazgo de la enzima que transfiere glucosa del uridina difosfato glucosa (UDPG) al glucógeno. Esta enzima fué encontrada primero en hígado (Leloir y Cardini, 1957; Leloir y Goldemberg, 1960) y un músculo (Leloir y colaboradores, 1959; Hauk y Brown, 1959; Villar Palasi y Larner, 1958). La reacción es la siguiente:

$$\text{UDPG} + \text{aceptor} \rightarrow \text{glucosil} - \alpha(1 \rightarrow 4)\ \text{aceptor} + \text{UDP}$$

Esta enzima puede llamarse glucógeno sinteasa o si no con un nombre más correcto pero más largo: UDPG-glucógeno transglucosilasa. El esquema general de la síntesis del glucógeno sería el que aparece en la figura 1.

En muchas de sus propiedades la glucógeno sinteasa se asemeja a la fosforilasa. Es sabido que esta última sólo puede agregar unidades de glucosa a ciertos aceptores. La reacción va a la máxima velocidad cuando el aceptor es glucógeno, más lenta con glucógeno degradado y no tiene lugar con oligosacáridos. La glucógeno sinteasa requiere un aceptor del mismo tipo del que necesita la fosforilasa.

Cuando la fosforilasa agrega una unidad de glucosa al glucógeno se forma una nueva unión α 1 $\rightarrow$ 4. Esto es también el caso con la glucógeno sinteasa.

Pero hay una diferencia fundamental entre la formación de glucógeno a partir de glucosa-1-fosfato y del UDPG. Esta diferencia reside en el equilibrio de la reacción. Mientras que con la fosforilasa el equilibrio se desplaza fácilmente hacia la degradación del glucógeno, con la sinteasa el equilibrio está al parecer completamente desplazado hacia la síntesis. Tan es así que no se ha podido revelar la reversibilidad de

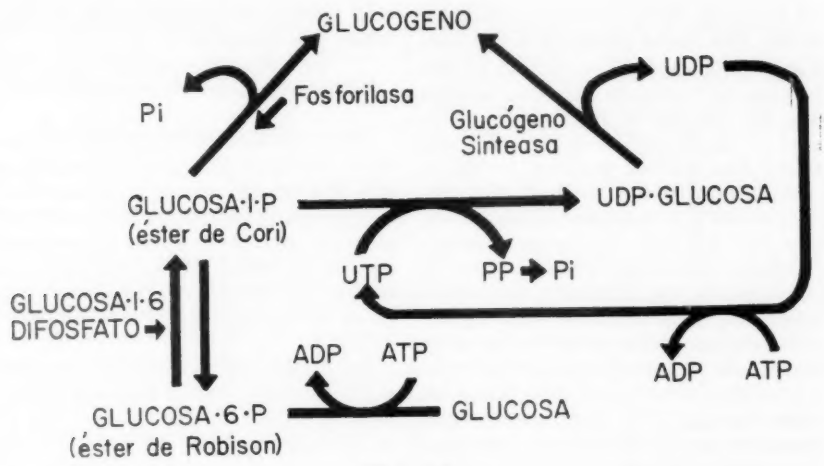

Figura 1

la reacción. Por lo tanto desde el punto de vista termodinámico la glucógeno sinteasa es más adecuada para efectuar síntesis que la fosforilasa. Otra característica interesante de la glucógeno sinteasa es la de ser fuertemente activada por el glucosa-6-fosfato. La concentración de éste que produce la mitad de la velocidad máxima es alrededor de 6 x 10^{-4}M. El glucosa-1-fosfato no produce activación así como tampoco muchas otras substancias que han sido ensayadas. El glucosamina-6-fosfato y galactosa-6-fosfato tienen un efecto similar al del glucosa-6-fosfato aunque algo menos intenso.

El mecanismo por el cual el glucosa-6-fosfato actúa como activador de la glucógeno sinteasa es difícil de comprender. Se ha observado que cuando se incuba la enzima con glucógeno y UDPG marcado en la glucosa con C^{14} se transfiere la radioactividad al glucógeno. Pero usando glucosa-6-fosfato marcado no aparece radioactividad en el glucógeno. Se han realizado muchos experimentos destinados a detectar la formación de un disacárido fosfato como intermediario en la reacción, pero ninguno de ellos ha tenido éxito.

La glucógeno sinteasa sólo puede formar uniones $\alpha\ 1 \rightarrow 4$, o sea que sólo puede alargar las ramas externas del glucógeno. Para que se forme un polisacárido ramificado es necesaria la acción de otra enzima que se ha llamado enzima ramificante cuando se la obtiene de tejidos animales y enzima Q cuando se la obtiene de vegetales (Cori y Cori, 1943; Larner, 1953). Su acción parece consistir en la transferencia de un trozo de cadena $\alpha\ 1 \rightarrow 4$ a otra cadena formando una unión $\alpha\ 1 \rightarrow 6$. Para que se produzca esta transferencia es necesario que la cadena externa llegue a tener unas 11 a 20 unidades.

Degradación del glucógeno

Cuando la fosforilasa actúa sobre el glucógeno en el sentido de la degradación, se van eliminando unidades de glucosa hasta llegar cerca de una unión $\alpha\ 1 \rightarrow 6$. Cuando quedan en término medio unas 4 unidades por rama exter-

na la acción de la fosforilasa cesa. La acción sólo puede proseguir si se hidrolizan las uniones α 1 → 6. Este último proceso es catalizado por una enzima que ha recibido el nombre de amilo α (1 → 6) glucosidasa (Cori y Larner, 1951). Esta enzima produce la liberación de una molécula de glucosa por cada unión α 1 → 6, por un mecanismo que aún no está totalmente aclarado (Walker y Whelan, 1959). Después de eliminada la rama lateral la fosforilasa puede continuar su acción hasta encontrarse con un nuevo obstáculo que a su vez puede ser eliminado por la amilo α (1 → 6) glucosidasa. Por lo tanto haciendo actuar a las dos enzimas sucesivamente se puede obtener información sobre el largo y número de ramas externas y este procedimiento ha permitido obtener valiosa información sobre la estructura del glucógeno.

Una enzima cuyo descubrimiento fué muy anterior a las mencionadas anteriormente es la α-amilasa. Su presencia en saliva, páncreas y plasma sanguíneo es bien conocida pero en general se admitía que no existe en músculo e hígado. Sin embargo aunque se tomen precauciones para eliminar la sangre por perfusión siempre queda cierta actividad de α-amilasa en los tejidos. Su acción consiste en hidrolizar uniones α 1 → 4 al azar siendo los productos finales oligosacáridos, maltotriosa y maltosa.

Un papel que se puede atribuir a la α-amilasa en los tejidos es el de crear núcleos para la formación de nuevas moléculas de glucógeno. La glucógeno sinteasa sólo puede agrandar moléculas de glucógeno pre-existentes. Para crear una nueva molécula resultaría necesario ya sea dividir en partes una preexistente o si no formarla a partir de monosacáridos.

Otra enzima que se ha encontrado en los tejidos animales transfiere unidades de glucosa de una molécula a otra (Miller y Copeland, 1958; Stetten, 1959), por ejemplo:

2 maltosa → maltotriosa + glucosa

Actúa también con oligosacáridos mayores y con el glucógeno. Su papel es aún desconocido pero se le atribuye una función en la formación de nuevos núcleos de glucógeno como se mencionó para la α-amilasa. En las plantas se ha encontrado una enzima similar y se la llama D enzima (Peat y colaboradores, 1953).

Del hígado (Sie y colaboradores, 1958) y del músculo (Beloff-Chain y colaboradores, 1955) se pueden extraer una serie de oligosacáridos de 2, 3, 4 y más unidades de glucosa. No se conoce con certeza cuál es el mecanismo de su formación ni su función fisiológica. Substancias iguales se forman por acción de la α-amilasa o de la transglucosidasa.

Formación de glucógeno a partir de la galactosa

Los estudios sobre el metabolismo de la galactosa están íntimamente relacionados con los realizados sobre la formación de glucógeno porque fué a consecuencia de una investigación sobre el metabolismo de la galactosa en una levadura que se descubrió el UDPG (Caputto y colaboradores, 1950), el que interviene como factor fundamental en la formación del glucógeno. El primer paso en la utilización de galactosa en tejidos animales es la formación de galactosa-1-fosfato (Kosterlitz, 1943) de acuerdo a la ecuación siguiente (Trucco y colaboradores, 1948)

ATP + galactosa →
ADP + galactosa-1-fosfato (1)

Es en la ulterior transformación del galactosa-1-fosfato en que interviene el

UDPG por un mecanismo que se puede resumir en las ecuaciones siguientes:

$$\text{Galactosa-1-fosfato} + \text{UDPG} \rightleftharpoons \text{glucosa-1-fosfato} + \text{UDP-Galactosa} \quad (2)$$

$$\text{UDP-galactosa} \rightleftharpoons \text{UDPG} \quad (3)$$

Suma:

$$\text{galactosa-1-fosfato} \rightleftharpoons \text{glucosa-1-fosfato}$$

La reacción 2 consiste en una transferencia de uridina monofosfato del UDPG al galactosa-1-fosfato que da por resultado la formación de los productos que aparecen en la parte derecha de la ecuación. La enzima que cataliza dicha reacción ha sido denominada galactosa-1-fosfato uridil transferasa (Maxwell y colaboradores, 1955).

La reacción 3 consiste en el cambio de posición del hidroxilo en 4 de la galactosa: el carbono 4 en ésta tiene la disposición espacial I y el de la glucosa tiene la disposición II.

La enzima correspondiente se denomina UDPG-4-epimerasa, ha sido hallada por Leloir (1951) y estudiada por Kalckar y sus colaboradores (cf. Kalckar, 1958). Ellos descubrieron que la reacción requiere cantidades catalíticas de difosfopiridina nucleótido, pero no han podido hasta ahora aclarar su mecanismo íntimo.

En la enfermedad congénita llamada galactosemia hay una falla en la utilización de la galactosa. Esta falla consiste en la ausencia de la galactosa-1-fosfato uridil transferasa, es decir de la enzima que cataliza la reascción 2. Las otras enzimas específicas para la galactosa o sus derivados (reacciones 1 y 3) tienen actividad normal.

El camino que sigue la galactosa para llegar a glucógeno sería el que aparece en la figura 2.

También existe otra vía por la cual la galactosa puede llegar a glucógeno. Hay una enzima que cataliza la reacción:

$$\text{UTP} + \text{galactosa-1-fosfato} \rightarrow \text{UDP-galactosa} + \text{PP}$$

Esta enzima, UDP-galactosa pirofosforilasa fué estudiada por Isselbacher (1958) y sería la responsable de la pequeña utilización de galactosa en los galactosémicos. Su importancia cuantitativa es pequeña ya que sólo sería responsable de un 5 % de la galactosa utilizada normalmente (Anderson y colaboradores, 1957). La vía de transformación de galactosa a glucógeno a través de la UDP-galactosa pirofosforilasa sería la que se muestra en la figura 3.

Siu y Wood (1959) han hecho un estudio comparativo de la formación de glucógeno a partir de glucosa y de galactosa marcadas en el carbono 2 con C^{14}. Luego aislaron el glucógeno y determinaron el C^{14} en las distintas posiciones de la glucosa obtenida por hidrólisis. Observaron que cuando suministraban galactosa el 90 a 93 % del C^{14} aparecía todavía en posición 2 en la glucosa del glucógeno. En cambio cuando se suministraba glucosa quedaba un 68 a 80 % del C^{14} en posición 2 mientras que el resto aparecía en posiciones 1, 5 y 6 principalmente. El pasaje del C^{14} de la posición 2 a las otras probablemente tiene lugar principalmente por ruptura de la cadena cuando la hexosa está en el estado de fructosa mono o difosfato. Por lo tanto la conclusión que se puede sacar de los experimentos antes mencionados es que en el camino de la galactosa no hay una equilibración tan rápida con los fructosa-fosfatos como en el camino de la glucosa.

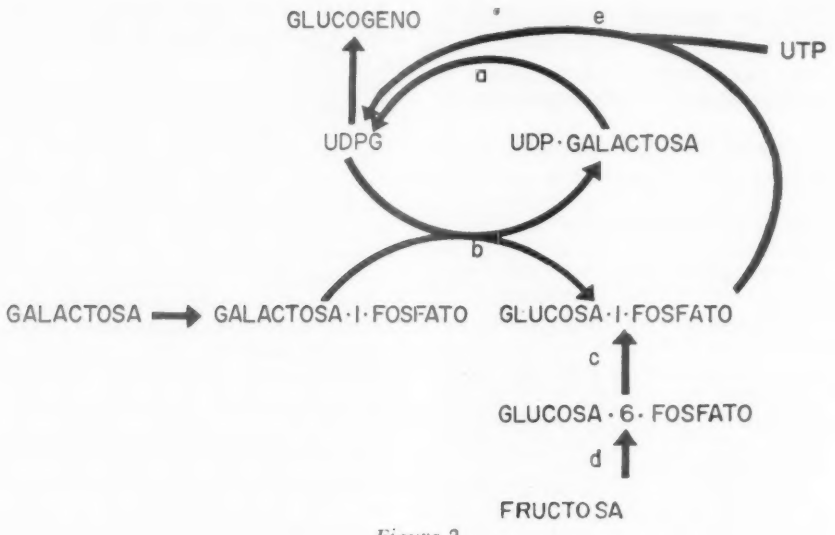

Figura 2

Para la galactosa la posibilidad de equilibración sería sucesivamente a través de las reacciones a, b, c, d en la figura 2 y luego d, c, e.

En cambio para la glucosa el esquema sería:

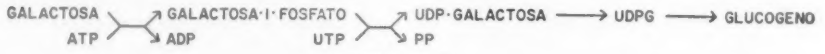

Figura 3

glucosa → glucosa-6-fosfato → glucosa-1-fosfato → UDPG → glucógeno
↓
fructosa-6-fosfato

Aquí se ve que para llegar de glucosa a fructosa-fosfato sólo se requieren dos pasos, y por lo tanto, que es más probable una equilibración rápida con esta última. Si la formación de glucógeno a partir de la galactosa tuviera lugar a través de glucosa-1-fosfato es probable que no se hubieran observado las diferencias entre los dos azúcares que aparecieron en los experimentos de Siu y Wood.

Síntesis de UDPG

La formación de glucógeno requiere un aporte constante de UDPG. El mecanismo de síntesis de esta substancia, que se conoce gracias a los trabajos de Kalckar y otros (cf. Kalckar, 1958), es el siguiente:

Uridina trifosfato + glucosa-1-fosfato
UDPG + pirofosfato inorgánico

El nombre común de la enzima correspondiente es: UDPG-pirofosforilasa.

A su vez el UTP puede formarse por transfosforilación con cualquiera de los otros nucleósidos trifosfatos. Por ejemplo:

$$ATP + UDP \rightarrow UTP + ADP$$

Por otra parte es sabido que ciertas reacciones de la glucolisis, de la respiración y de la fotosíntesis dan lugar a la formación de ATP.

Regulación fisiológica

Los trabajos de Sutherland y colaboradores (cf. Sutherland, 1951) han demostrado que la actividad de la fosforilasa está sometida a un delicado control fisiológico. En el hígado la fosforilasa se encuentra en dos formas, una activa y otra inactiva. Ambas formas son interconvertibles en la forma siguiente:

$$\text{Fosforilasa inactiva} \underset{-P_i}{\overset{+P_i}{\rightleftharpoons}} \text{fosforilasa activa}$$

La reacción hacia la derecha es catalizada por una enzima que transfiere fosfato del ATP a la fosforilasa inactiva. En la reacción hacia la izquierda interviene una fosfatasa que libera fosfato inorgánico. La formación de fosforilasa activa es acelerada por un mecanismo desconocido por el 3'-5'-adenosina monofosfato.

La formación de este último a partir del ATP es a su vez acelerada por la adrenalina o por el glucagon. Es en esta forma indirecta que estas últimas substancias determinan un aumento de fosforilasa activa y con ello un aumento de la glucogenolisis.

Patología

Los nuevos conocimientos permiten comprender mejor algunas de las alteraciones del metabolismo del glucógeno en el hombre. Desde que fué descripto el sindrome de Von Gierke en 1929 se han realizado muchos estudios y se ha llegado a la conclusión de que existen varios tipos distintos de enfermedad con almacenamiento anormal de glucógeno (cf. Cori, 1952-53; Andersen, 1954).

En algunos casos de acumulación de glucógeno en el hígado se halla una gran disminución de la glucosa-6-fosfatasa. Esto llevaría a un aumento de glucosa-6-fosfato y de glucosa-1-fosfato. Siendo el glucosa-6-fosfato un activador de la glucógeno sinteasa se produciría un aumento de actividad de ésta. Por otra parte el aumento de glucosa-1-fosfato disminuiría la degradación provocada por la fosforilasa y aumentaría la síntesis del UDPG por la UDPG-pirofosforilasa. Por lo tanto habría tres factores sinérgicos tendientes a aumentar el contenido del glucógeno.

Se han descripto varios otros tipos de acumulación patológica del glucógeno. El tipo I que afecta al hígado y riñón es el ya mencionado. En el tipo II la acumulación se produce especialmente en el corazón, lengua y cerebro, no habiéndose podido averiguar cuál es la alteración enzimática.

En los otros dos tipos está especialmente afectado el hígado y se han podido descubrir anomalías en la composición del glucógeno. El glucógeno de los enfermos de uno de los grupos se diferencia del normal por tener las ramas externas más largas y por lo tanto se cree que ello es debido a una disminución en la actividad de la enzima ramificante. En cuanto al glucógeno del otro grupo tiene las ramas externas más cortas que lo normal y se ha sugerido que en estos casos estaría disminuida la amilo-1,6-glucosidasa.

Se conocen algunas miopatías caracterizadas por fácil fatigabilidad que mejora por administración de glucosa o de lactato. En los músculos de estos enfermos, el glucógeno se encuentra en con-

centración normal o ligeramente aumentada y la valoración de las enzimas ha demostrado que la fosforilasa se encuentra ausente, mientras que las otras enzimas del metabolismo de los hidratos de carbono, incluyendo la glucógeno sinteasa, están en concentración más o menos normal (Larner y Villar-Palasi, 1959; Mommaerts y colaboradores, 1959; Schmid y colaboradores, 1959; Hauk y colaboradores, 1959).

Esto demuestra evidentemente que en estos casos el glucógeno debe sintetizarse por un camino distinto al de la fosforilasa y, además, explica la aparición del síntoma más marcado de esta enfermedad. El músculo para realizar su trabajo utiliza principalmente la energía derivada de la combustión del glucosa fosfato que proviene normalmente de la glucosa libre por vía de la hexoquinasa y del glucógeno vía de la fosforilasa. Al no existir fosforilasa sólo habría una provisión parcial de glucosa fosfato y por eso se produciría rápidamente la fatiga.

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CHANGES IN THE CONCENTRATION OF ASCORBIC ACID INDUCED BY ESTRADIOL ON ENDOCRINE GLANDS OF MALE RATS *

JAIME A. MOGUILEVSKY, HÉCTOR N. TORRES, GRATO E. BUR, AARÓN N. ERENFRYD AND MANUEL R. MALINOW
(Institute of Physiology, Buenos Aires Medical School, Buenos Aires.)

ALTHOUGH the concentration of ascorbic acid is high in several endocrine glands, little is known concerning its possible rôle; nevertheless, that the vitamin is somewhat connected with cellular metabolism is apparently attested by the fact that its concentration varies under several circumstances. Adrenal ascorbic acid, for instance, decreases following ACTH administration (1) or hypophysectomy (2), and its pituitary concentration is decreased under unspecific stressful conditions (3). Ascorbic acid is also very high in the testicles (4) but, as far as we are aware, no studies have related its concentration with functional modifications of the glands. In the present paper, the changes brought about by estradiol on the ascorbic acid content of several endocrine glands of male rats, are reported.

MATERIAL AND METHODS

Adult white male rats, of the Williams strain from the Institute of Physiology, on standard laboratory diet, were used. Four groups of 12 rats each were studied: 1) intact controls; and animals injected with 2) olive oil; 3) 17 α-estradiol, or, 4) 17 β-estradiol. The hormones were dissolved in olive oil at a concentration of 75 μg/ml; the animals received 1 ml intramuscularly twice a week during four weeks. The rats were weighed weekly. At the end of the experimental period, the animals were killed by decapitation and the adrenals, hypophysis and testicles rapidly removed, blotted with filter paper, weighed on a torsion balance to the nearest mg and immersed in trichloroacetic acid. Ascorbic acid was then determined accordingly with Roe and Kuether's method (5), as modified by Sayers, Sayers and Woodbury (6). Results were compared with Student's "t" test accordingly with Fisher's tables (7). In three animals the organs were fixed in Bouin's solution (8), embedded in paraphine, stained with haematoxylin-eosin, Mallory trichrome stain and P.

(*) This work was partly supported with a grant from the "Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina".

A.S. Mc Manus'method ([8]), and studied microscopically.

Results

In Table 1, the percentage body weight changes as well as weight of the adrenal, pituitary and testicular glands are reported; Table 2 shows the same endocrines expressed per 100 g of body weight. As can be seen in Table 3, neither the injection of the vehicle (olive oil) nor of the 17 α-isomer of estradiol, modified the concentration of ascorbic acid in the adrenals, hypophysis or testicles. The administration of 17 β-estradiol, on the contrary, was able to increase the concentration of the vitamin in the adrenals, while decreasing, at the same time, its concentration in the testicles. The total amount of ascorbic acid was increased by 17 α-estradiol in the adrenals, possibly because of a slight, non-statistically significant, increase brought about in the total weight of these glands.

Histological changes were seen only in the animals injected with 17 β-estradiol: a cytological hypertrophy was observed in the adrenal glands, the cells showing a vacuolated cytoplasm with decreased lipid content; an increased vascularization of the pituitary gland with a greater basophilic content was also present in these animals. The testicles showed an arrest of spermatogenesis up to the spermatid stage; cellular desquamation was common and no spermatozoids were seen. The Sertoli's cells were somewhat vacuolated with hypertrophic nuclei and the interstitial cells of Leydig were atrophic.

Discussion

Changes in the ascorbic acid pituitary content induced by estrogens, have been studied histochemically, mainly by Giroud ([4]) and by Mancini and Pasqualini ([9]). These authors found an increase within the acidophilic and chromophobe cells, and a decrease within the basophilic cells; such opposite changes were not detected with the present chemical method.

A histochemical decrease of ascorbic acid within the cortical cells and around the capillary sinusoids of the adrenal glands, induced by estradiol dipropionate in rats, has been reported ([10]). Our experiments show that an oil solution of 17 β-estradiol is able to increase the vitamin content of the adrenals, while neither the vehicle nor an isomer of the hormone, brought about any changes. Since estrogens may show different effects in the adrenal glands of several species, our findings must be confirmed with further studies in other animals over a wider dosage range.

Finally, the decreased vitamin content found by us in the testicles of the 17 β-estradiol-injected animals, are apparently well correlated with the histologically demonstrated atrophy of the gland; these functional relationships as well as the possible significance of ascorbic acid in the ejaculated seminal fluid, seems worth of further study.

Summary

Ascorbic acid has been chemically determined in the pituitary, adrenal and testicular glands of male rats under estradiol therapy. The hormone, but not the oil vehicle nor its 17 α-isomer, was able to decrease the vitamin concentration in the testicles and to increase its concentration in the adrenal glands. No changes were detected in the hypophysis. Possible functional relationships between the vitamin and the testicles, are discussed.

TABLE I

Weight of endocrines and body weight changes of male rats (•)

GROUP	Numb. of animals	ADRENALS (mg)	HYPOHYSIS (mg)	TESTICLES (mg)	Percentage of weight changes in 4 weeks
1) Intact controls	12	23,88 ± 1,274	5,250 ± 0,500	2,109 ± 0,039	34,171 ± 2,213
2) Oil-injected control	12	23,12 ± 1,274	5,000 ± 0,801	2,075 ± 0,093	29,061 ± 2,183
3) 17 α-Estradiol	12	27,30 ± 2,423	6,370 ± 0,677	2,280 ± 0,161	44,446 ± 1,224
4) 17 β-Estradiol	12	32,50 ± 4,182	7,120 ± 0,731	1,208 ± 0,153	21,513 ± 2,254
"t" 1 vs 2		0,429	0,265	0,038	1,650
"t" 2 vs 3		1,555	1,318	1,114	6,297 (***)
"t" 2 vs 4		2,186 (**)	1,961 (*)	4,870 (***)	2,387 (**)
"t" 3 vs 4		1,106	0,763	4,963 (***)	9,426 (***)

(•) Mean ± standard error $\left(Se = \sqrt{\frac{\Sigma d^2}{n(n-1)}}\right)$

(*) $p < 0.1$
(**) $p < 0.05$
(***) $p < 0.01$

TABLE II

Relative weight of endocrines in male rats (•)

GROUP	Number of animals	ADRENALS (mg/100 g)	HYPOPHYSIS (mg/100 g)	TESTICLES (mg/100 g)
1) Intact controls	12	13,83 ± 0,06	3,061 ± 0,099	1,267 ± 0,028
2) Oil-injected controls	12	14,05 ± 1,85	3,406 ± 0,132	1,310 ± 0,141
3) 17 α-Estradiol	12	12,55 ± 1,97	3,623 ± 0,081	1,326 ± 0,045
4) 17 β-Estradiol	12	20,66 ± 0,70	5,412 ± 0,199	0,731 ± 0,060
"t" 1 vs 2		0,674	2,103 (*)	0,300
"t" 2 vs 3		0,555	1,409	1,048
"t" 2 vs 4		3,333 (**)	8,428 (***)	3,780 (***)
"t" 3 vs 4		3,880 (***)	8,359 (***)	7,885 (***)

(•) Mean ± standard error.
(*) $p < 0.05$
(**) $p < 0.02$
(***) $p < 0.01$

TABLE III

Ascorbic acid content (in mg) in endocrines of male rats (•)

GROUP	Numb. of animals	ADRENALS		HYPOPHYSIS		TESTICLES	
		Total	A.A./100 g (••)	Total	A.A./100 g	Total	A.A./100 g
1) Intact controls	9	96,4 ± 4,44	405 ± 13,0	13,9 ± 2,93	221 ± 17,8	599 ± 23,6	26,5 ± 1,21
2) Oil-injected control	9	71,1 ± 12,5	348 ± 29,2	9,87 ± 1,41	163 ± 22,9	511 ± 35,2	25,9 ± 1,18
3) 17 α-Estradiol	9	115,7 ± 14,1	378 ± 27,5	22,1 ± 9,50	157 ± 27,30	571 ± 40,3	21,8 ± 1,36
4) 17 β-Estradiol	9	147 ± 10,1	459 ± 17,6	11,8 ± 2,31	208,5 ± 33	276 ± 37,1	20,1 ± 1,73
"t" 1 vs 2		1,992	1,803	1,000	2,001	0,340	0,351
"t" 2 vs 3		2,404 (*)	0,750	1,282	0,173	1,114	0,611
"t" 2 vs 4		4,865 (***)	3,204 (***)	1,319	1,137	4,667 (***)	3,264 (***)
"t" 3 vs 4		1,819	2,442 (*)	0,105	1,230	5,440 (***)	2,200 (*)

(•) Mean ± standard error.
(••) A.A., ascorbic acid.
(*) $p < 0.05$
(***) $p < 0.01$

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PRODUCTION OF A PEPSINTENSIN-LIKE SUBSTANCE WITH TOAD PLASMA

ALEJANDRO C. PALADINI AND MARCELO N. MUNTAABSKI
(Chair of Biological Chemistry, Ist. Part, School of Pharmacy and Biochemistry and Institute of Physiology, School of Medicine, University of Bs. As.)

THE EXISTENCE of the renin-angiotensinogen system has been proved in man, horse, hog, ox, sheep, rat, and chicken. In ducks, the injection of chicken renin elicits a positive blood pressure response, indicating the presence of angiotensinogen in this species.

In all poikilotherms tested (sharks, toads and some snakes), no evidence for the renin-angiotensin system has been found. Bean [1], who collected most of this data, points to renin as the most likely missing link.

In 1942, Croxatto and Croxatto [6] found that pepsin acting on bovine plasma globulins produced a polypeptide: pepsitensin, which is pharmacologically and chemically very similar to angiotensin. Later on, Braun Menéndez and col. [2, 3], showed that angiotensinogen and pepsitensinogen, were probably identical.

We have studied the action of pepsin on toad (*Bufo arenarum Hensel*) plasma, to determine whether pepsitensin could be formed in this poikilotherm.

MATERIAL AND METHODS

Toads of 100 to 200 g were used. Under ether anesthesia they were heparinized through the abdominal vein and bled through the aorta. The plasma separated by centrifugation was kept in the freezer. All the experiments were done between February and October. Crystalline pepsin was obtained from Armour or Worthington.

One ml of plasma, acidified to pH 6.2 or 3.5 with 1 M hydrochloric acid, was additioned with 0.1 ml of a pepsin solution containing 30 mg of enzyme per ml and adjusted to the corresponding pH. The mixture was incubated 10 minutes at 38°C, adjusted to pH 4.5 and heated 5 minutes in a boiling water bath. After centrifugation the pressor activity of the neutralized supernatant, was measured in nephrectomized rats anesthetized with Nembutal [9] (*).

Plasma incubated without pepsin and submitted to the same procedure was used as a blank in all instances. Pepsin itself repeatedly proved to give no vasoactive substances.

The Läwen-Trendelenburg prepara-

(*) One rat unit is the pressor activity of 0.2 ml of a standard angiotensin preparation and is equivalent to approximately, 0.014 Goldbladtt unit and to 0.057 μg of noradrenaline.

tion was done according to Rapela [10].

Paper electrophoresis was carried out on Whatman Nº 3 paper, using a tris-(hydroximethyl)-aminomethane-barbital buffer, pH 8.6 of the following composition: tris-(hydroxymethyl)-aminomethane: 45 g, sodium barbital: 16 g, barbital: 2.2 g, glacial acetic acid: 13.0 g, water to 1000 ml [11]. The paper was placed between glass plates, submitted to a pressure of 1 kg per cm^2. The potential drop was 30 volt per cm, during 2 hours. The whole experiment was carried out in the cold room at 0°C. At the end of the run a guide strip was dyied with bromophenol blue according to Durrum [7], as modified by Kunkel and Tiselius [8]. Quantitative estimation of the stained proteins was done following Cremer and Tiselius [5].

Results

Table 1 shows the results obtained in some of the experiments performed. The plasma not treated with pepsin (a), has a small amount of pressor activity but this increases sharply on incubation with pepsin (b).

These results can be interpreted in the sense that pepsin acting on a plasma protein, releases one or more thermostable pressor active substances.

It should be noticed that pepsin is able to catalize the reaction equally well, or perhaps better, at pH 6.2 than at pH 3.5. The same fact was found by Braun Menéndez and Paladini [3], studying the action of pepsin on horse and ox plasma.

Figure 1 shows the action of the incubated plasma on the Läwen-Trendelenburg preparation. The blank, in these experiments, was done incubating the plasma with pepsin previously destroyed by alkali. The vasoconstriction produced by the sample incubated with active pepsin is clearly evident.

Our preparations were also tested to ascertain if they had any effect on the blood pressure of the intact toad. We had no positive results, but as the animals were also relatively insensitive to high dosis of angiotensin, and our test solutions were relatively dilute, no definite conclusions can be reached.

In order to identify the chemical nature of the active substance (or substances), the following tests were performed:

Action of Trypsin:

Incubation of our samples with 0.5 mg per ml of crystalline trypsin during 30 minutes at 38°C and pH 7.5 completely destroyed the biological activity. Under analogous conditions angiotensin is similarly destroyed.

These results strongly suggest the polypeptide nature of the active substances. The pressor activity of some plasmas before pepsin treatment was also susceptible to trypsin digestion.

Alkali stability:

Table 2 shows the well known fact that adrenaline is alkali-labile. Under the mild conditions of the test performed adrenaline is the only pressor substance destroyed.

This result, together with the action of trypsin, discards adrenaline as the substance responsible for the observed pressor activity.

Acid stability:

Table 3 shows the results of heating at 100°C, in 1 M hydrochloric acid, angiotensin and the toad plasma pressor substance/s.

The figures obtained demonstrate the striking parallelism between the rate of destruction of both substances.

TABLE I

Pressor activity of toad plasma treated with pepsin.

EXP. Nº	PRESSOR ACTIVITY rat U/ml		△ (b - a)	pH of INCUBATION
	PLASMA (a)	PLASMA PEPSIN (b)		
1	0	10	10	
2	4	15	11	
3	8	27	19	
4	3	18	15	
5	3	22	19	6.2
6	3	14	11	
7	2	11	9	
8	0	8	8	
9	1	17	16	
AVERAGE:	2.6 ± 2.5 (*)	15.7 ± 6.1	13.1 ± 4.8	
10	1	12	11	
11	0	14	14	
12	6	17	11	
13	3	6	3	3.5
14	1	12	11	
15	6	11	5	
AVERAGE:	2.8 ± 2.6	12.0 ± 3.6	9.1 ± 4.2	

(*) Standard error.

TABLE II

SUBSTANCE	Pressor Activity RAT U/ml	
	0 min	30 min
ADRENALINE	8.8	0
ANGIOTENSIN	5.0	4.7
PLASMA + PEPSIN	6.3	6.2
PLASMA	1.6	1.3

ALKALI STABILITY OF TOAD PLASMA PRESSOR SUBSTANCE

The samples made 0.02 M with sodium hydroxide were incubated at 38°C for the stated amount of time. The assay was carried out with the samples previously neutralized.

TABLE III

SUBSTANCE	Pressor Activity Measured In the Rat. Percentage values		
	0 min	15 min	60 min
ANGIOTENSIN	100	70	30
PLASMA + PEPSIN	100	70	32

ACID STABILITY OF TOAD PLASMA PRESSOR SUBSTANCE

The samples were incubated at 100°C in 1 M hydrochloric acid for the stated amount of time. The assay was carried out with the samples previously neutralized.

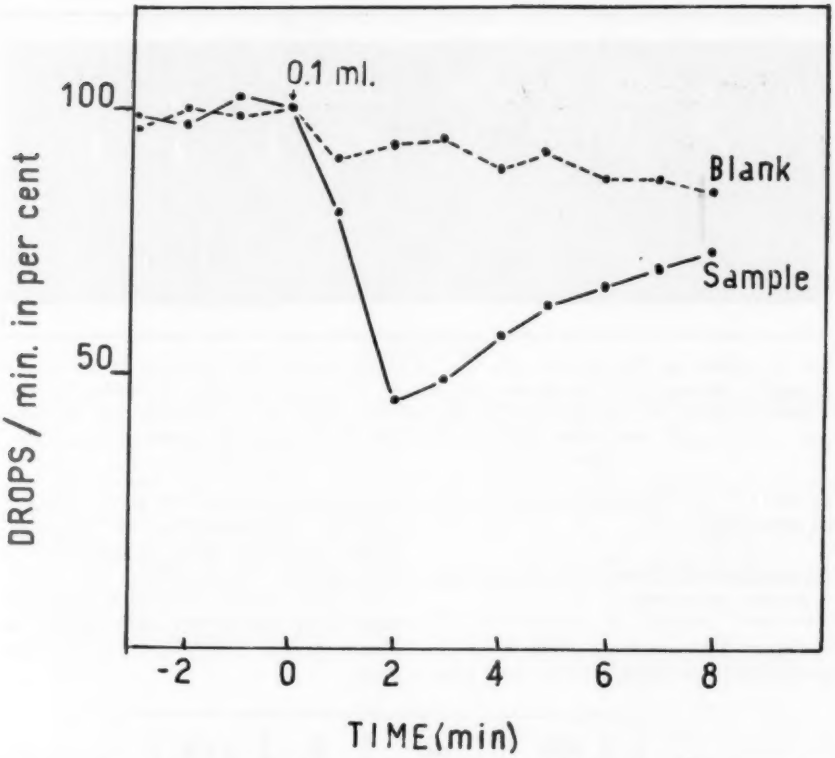

FIG. 1.—*Effect of Toad Plasma Pressor Substance on the Läwen-Trendelenburg preparation. The incubated plasma had a pressor activity of 3.7 rat units per ml. The blank, with no pressor activity, was prepared with the same plasma incubated with pepsin previously destroyed by alkali. The points represent average values from three experiments. Abscissae, drops/min in per cent of initial values.*

Action of Thioglycollic acid:

The pressor substance/s from toad plasma are not affected by incubation with thioglycollic acid under the conditions described in figure 2.

The blood pressure tracings obtained in a rat injected with equal volumes of identically treated samples of angiotensin, vasopressin (Pitressin), and toad plasma pressor substance/s show the similar behavior of angiotensin and the substance/s under investigation.

Extractability with sec-Butanol:

Angiotensin is easily extracted from aqueous solutions by sec-butanol at pH 1 in the presence of sodium chloride ([4]).

In several experiments it was found that the substance/s under investigation behaved similarly to angiotensin also in this respect. Solutions of angiotensin or equipressor amounts of toad plasma pressor substance/s in 0.1 M hydrochloric acid plus 15 per cent of sodium chloride, were totally extracted

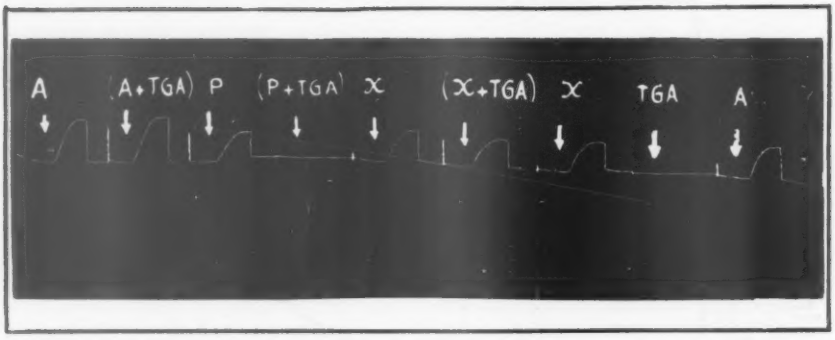

FIG. 2.—*Action of Thioglycollic acid. 0.3 ml of the sample plus 0.003 ml of Merthiolate (4 mg/ml), and 0.03 ml of thioglycollic acid (0.2 M) or 0.03 ml of saline were incubated during 2 hours at 38°C (pH: 7.5), previously to the injection to the rat. A: angiotensin; P: pitressin; X: toad plasma plus pepsin; TGA: thioglycollic acid blank.*

by three extractions with equal volumes of sec-butanol.

Electrophoretic identification of active plasma proteins:

Figure 3 shows the results of an experiment in which 0.2 ml of toad plasma were submitted to paper electrophoresis fractionation, as described under Methods, on a Watman Nº 3 filter paper, 11 cm. wide. Seven fractions were eluted from the unstained paper and assayed for their ability to release pressor activity on pepsin treatment.

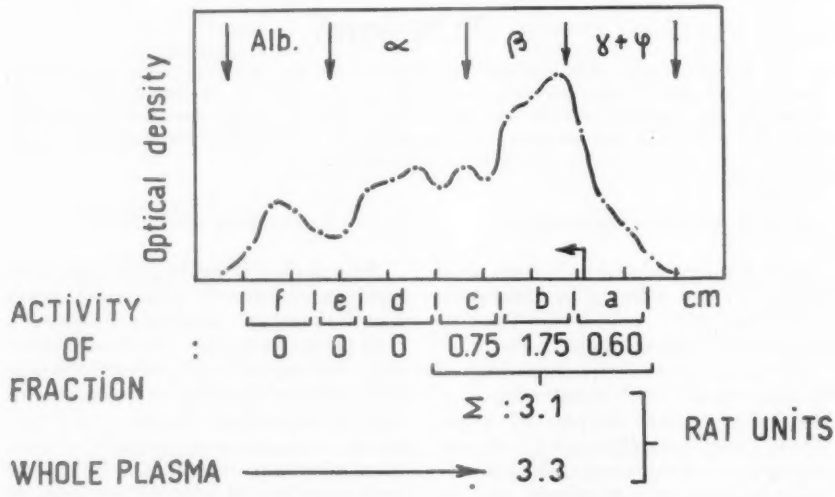

FIG. 3.—*Electrophoretic Identification of Active Plasma Fractions. For the procedure, see text.*

It can be seen that the precursor of the substance/s in study has the mobility of a β-globulin. The high recovery of activity from that electrophoretic fraction, further supports this result.

Summary and conclusions

Toad plasma on treatment with pepsin, is able to release one or more pressor polypeptides, which have many properties in common with angiotensin. Consequently, it may very well prove rewarding to look more carefully on the existence of renin in this animal.

One interesting finding, with possible general biological significance, is that β-globulins provide the substrate for this reaction, whereas in all examined mammals this role is played by α-globulins.

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FURTHER IMPROVEMENTS ON A METHOD FOR THE ESTIMATION OF ANGIOTENSIN IN BLOOD

OSCAR A. SCORNIK. ALEJANDRO C. PALADINI AND EDUARDO BRAUN MENÉNDEZ (†)

(Institute of Physiology, School of Medicine and Chair of Biological Chemistry, Ist. Part, School of Pharmacy and Biochemistry. University of Bs. As.

IN SPITE of continued research on experimental hypertension it has not been possible to establish with absolute certainty the participation of the renin-angiotensin system in the production of chronic nephrogenic hypertension. The indirect approach to this problem has failed to give any conclusive answer and measurements of renin in blood or in the kidney, or of angiotensin in blood, have not been specific or sensitive enough ([2, 3, 6, 7, 10, 11, 12]).

In 1951/52, Skeggs and his associates ([6, 10, 11]), established that in dogs with acute renal hypertension and in humans with malignant hypertension, there are increased amounts of circulating angiotensin. These facts have been objected since their method requires a large sample of blood (250 ml), the withdrawal of which may cause a drop in the systemic pressure and thereby liberate renin from the kidney ([4]).

Recently, though, we have been able to measure angiotensin in blood using a sample of only 50 ml. The method recovers practically all the angiotensin added "in vitro" and is satisfactorily sensitive and specific ([9]). More extensive use of this technic has shown the convenience of introducing several modifications that increase the reliability of the procedure. The present method as well as some experimental results obtained with it, are here reported.

(†) Deceased January 16, 1959.

PROCEDURE

Fifty ml of blood are collected directly into 200 ml of 96 per cent ethanol as previously described ([9]). The suspension is filtered in a buchner through a bed of Hyflo Supercel. The cake is carefully washed with 200 ml of 80 per cent ethanol. The collected filtrates are concentrated "in vacuo" (app. 30°C), until reduced to 130 ml. This volume is transfered to a separatory funnel, acidified with 2 ml of 5N hydrochloric acid and extracted once with 200 ml of ether. The aqueous phase is further concentrated to 50 ml "in vacuo". By carefully adjusting this solution to neutrality a heavy precipitate is formed and is discarded after centrifuging 10 minutes at 12000 g. The clear supernatant is

brought to pH 3.1 and if it is necessary, the procedure may be stopped at this stage by keeping the solution frozen at −15°C as long as required. The sample is then percolated through four 10 mm x 100 mm columns of Dowex 50 X-2, approximately 120 mesh, serially connected. The resin is prepared as previously described (9), but final equilibration is carried out at pH 3.1 with citrate buffer 0.2 N.

The rate of percolation is adjusted to be less than 0.3 ml per min.

The sample is washed in with 5 ml of water. When the top of the first column is dry, it is disconnected from the train and the second column is rinsed with a further 5 ml of water. This procedure is repeated until the four columns are disassembled. Each column is now eluted separately with 5 ml of 0.1 N sodium hydroxide, followed by 10 ml of water. The collected eluates are neutralized and centrifuged and the precipitate is discarded. The supernatant, concentrated in vacuo to 3 or 4 ml, is ready for biological assay and pharmacological identification.

If it is necessary to concentrate to smaller volumes, the excess of sodium chloride in the solution should be eliminated; this can be achieved by taking the sample to dryness and extracting the residue with glacial acetic acid. The filtered acid is then evaporated to dryness and the residue dissolved into a small volume (not less than 0.5 ml) of water.

The biological assay is carried out as previously described (9). Activity is expressed in rat units.

RECOVERY EXPERIMENTS OF ADDED ANGIOTENSIN: In five experiments, 10 rat units of standard angiotensin preparation (1), were added to 50 ml of blood; recovery of activity, after substracting the blank value of a similar sample without any addition, was 93.3 ± 1.2 (*) per cent.

(*) Mean ± standard error.

SENSITIVITY OF THE METHOD: The assay is able to measure pressor activity in solutions with more than 0.5 rat units per ml. If final volume of the sample is 0.5 ml it follows that the method can estimate down to 5 rat units per liter of blood (0.07 Goldblatt U/liter). Lower amounts may also be detected but quantitative assays or pharmacological identifications are not possible.

PHARMACOLOGICAL IDENTIFICATION: The samples are routinely tested for destruction on incubation with trypsin or thioglycollic acid, as previously described (9).

On two occasions, samples with pressor activity obtained from normal dogs were assayed for antidiuretic activity using the method of Jeffers et al. (5), with no positive results.

HYPOTENSIVE CONTAMINANTS: Some extracts injected into rats induce an initial hypotension which is followed by the typical hypertensive action. The substances responsible for this effect are not destroyed by trypsin nor are they extracted from its aqueous alkaline solutions by ether or chloroform or by n-butanol in acid medium. Routinely, we use the insolubility of these substances in butanol at low pH to remove their interference with the quantitative assay. Angiotensin is fairly well extracted under these conditions.

"IN VIVO" EXPERIMENTS: As an illustration of the method, measurements of blood levels of angiotensin in dogs under different experimental conditions, are reported.

Figure 1 shows the results of infusing angiotensin and renin into a nephrectomized dog. A male dog of 12.5 kg was bilaterally nephrectomized under ether anesthesia. 24 hours later it was anesthetized with nembutal (30 mg per kg), both vagi were cut and artificial respiration administered. Blood pressure was registered on a kymograph through a mercury manometer connec-

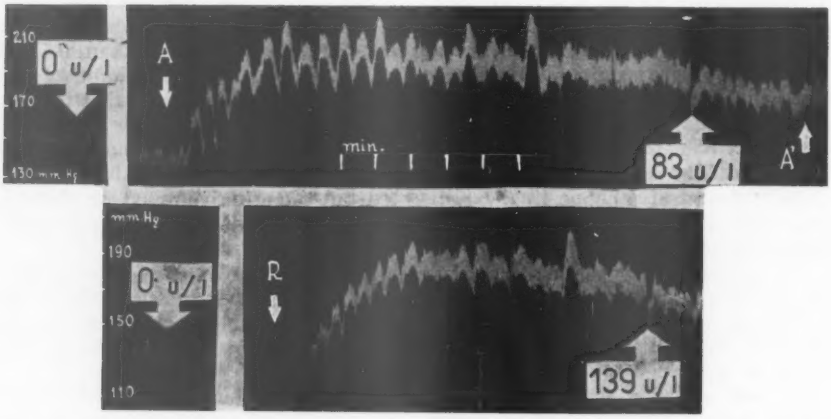

FIG. 1. — *Blood angiotensin levels in a nephrectomized dog before and after angiotensin infusion (A - A'), and renin injection (R). (For details, see text).*

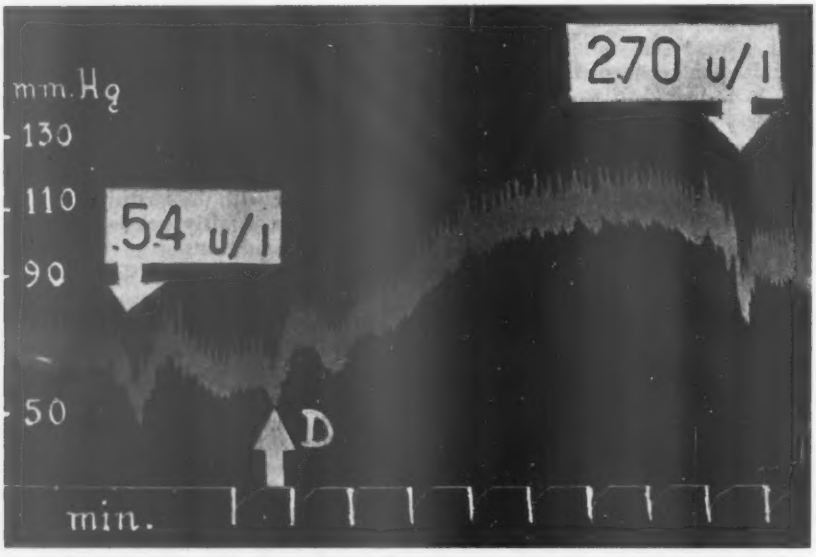

FIG. 2. — *Blood angiotensin levels in a dog during occlusion of both renal pedicles and after reestablishment (D) of the renal circulation (3 hours of complete bilateral ischemia). (For details, see text.)*

ted to the carotid artery. Injections were made in the jugular vein. Blood samples were taken from the femoral artery. As the figure shows, the initial sample had no pressor activity. Infusion of angiotensin was then started (fig. 1, A) at the rate of 1 ml per minute (135 rat units per ml). Fifteen minutes later a second blood sample was taken. Its pressor activity corresponded to 83 rat units of angiotensin per liter of blood. The infusion was stopped at A' and 20 minutes later a new sample of blood showed no circulating angiotensin. At R, 270 rat units of hog renin, prepared as indicated elsewhere ([8]), were injected; ten minutes later the angiotensin level in blood had risen to 139 rat units per liter.

Figure 2 shows the blood levels of angiotensin in a dog during and after the temporary occlusion of both renal pedicles. The renal arteries and veins of a female dog of 13.8 kg were clamped under nembutal anesthesia (30 mg per kg). The arterial pressure was registered as in the previous experiment. As shown in fig. 2, the blood level of angiotensin was 54 rat units per liter. At D, 3 hours after occlusion, the pedicles were released and an immediate rise in the arterial pressure followed. Seven minutes after D, the angiotensin level in blood was 270 rat units per liter.

The experiments shown in figures 1 and 2, demonstrate applicability of the method to measurements of exogenous angiotensin, endogenous angiotensin produced by exogenous renin and endogenous angiotensin produced by endogenous renin.

Summary

The method for estimating angiotensin in blood described by Paladini et al ([9]), has been modified to increase its reliability.

The results of its application to the measurements of blood levels of exogenous and endogenous angiotensin in dogs, are reported.

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WATER AND SOLUTE EXCRETION IN CHRONIC RENAL FAILURE. THE INFLUENCE OF POSTURAL CHANGES

MARIO F. VILLAMIL, CARLOS M. NIJENSOHN, IGNACIO J. DE LA RIVA, BLANCA N. BADANO AND ALBERTO C. TAQUINI
(Centro de Investigaciones Cardiológicas, Facultad de Ciencias Médicas, Azcuénaga 985, Buenos Aires, Argentina.)

ACCORDING to modern view the inability of diseased kidneys to concentrate the urine is not due to specific damage to the site where the concentrating mechanism takes place, but to osmotic diuresis. This was supported by the data of H. Smith et al. (1) who found $T^{c}_{mH_2O}$ per 100 ml of filtrate within normal limits in patients with renal failure of moderate degree under osmotic diuresis during the hydropenic state.

It is the purpose of the present study to investigate the role played by osmotic diuresis in the inability of damaged kidneys to dilute the urine. With the same purpose in mind, the influence of postural changes on water and solute excretion in chronic renal failure was also studied. As it is well known, change from recumbency to semivertical, standing or sitting positions is followed in normal people by increased proximal water and solute reabsorption. An opportunity was thus afforded to study in patients with chronic renal failure the capacity of proximal tubules to react to certain stimuli by increasing solute and water reabsorption and the influence of variable distal loads on the diluting capacity of the distal segments.

MATERIAL AND METHODS

Eleven patients with chronic renal disease of vascular (glomerulonephritis), infective (pyelonephritis) or congenital (polycystic kidneys) type were selected for study. Patients with chronic pyelonephritis had renal lithiasis and evidence of present or past urinary tract infection. Patients with chronic glomerulonephritis had a past history of hematuria and/or edema preceded by throat infection; in two of these cases the diagnosis was confirmed by biopsy. All patients had polyuria ranging from 2000 to 4500 ml per day and variable degrees of nitrogen retention.

The first 5 patients were studied under moderate water diuresis and the remaining 6 under maximal water diuresis. In experiments conducted under moderate water diuresis the subjects were made to drink at twenty minutes intervals 0.1 ml per kg weight per minute of a 1.4 ‰ sodium chloride solution throughout the experiment. Maxi-

mal water diuresis was attained through previous administration of 1200 ml of tap water and further replacement of urinary losses by an equivalent amount of a 1.4 ‰ sodium chloride solution.

Experiments were started after two hours equilibration in recumbency. Following a priming dose, an isotonic solution of mannitol with PAH in water was infused at constant rate throughout the experiment. After two periods in recumbency the subjects were tilted to 45° from the horizontal for two additional periods, the experiments being ended by two other periods in supine position.

At the end of each period the bladder was emptied through an indwelling multieyed catheter, residual urine being expelled by air. No washout fluid was used. Blood samples were simultaneously drawn from an antecubital vein through and indwelling needle. Plasma and urine samples were analysed for sodium, potassium, chloride, osmolality, mannitol and PAH. Sodium and potassium were determined by flame photometry (2), chloride by Scribner's modification of Schales and Schales' method (3), freezing point with a Beckmann thermometer, mannitol by Corcoran and Page's method (4) and PAH by Bratton and Marshall's method as modified by H. Smith et al. (5).

Calculations

Osmolar clearance (C_{OSM}) was calculated according to the formula $\frac{VU_{OSM}}{P_{OSM}}$ where V is the urine volume in ml per minute, U_{OSM} the osmolar concentration of urine and P_{OSM} the osmolar concentration of plasma. Free water clearance (C_{H_2O}) was calculated from urine volume and osmolar clearance according to the formula $V\text{-}C_{OSM}$ (6) which may also be written $(1-\frac{U_{OSM}}{P_{OSM}})\,V$. Sodium and chloride reabsorption was calculated from excretion and filtered load corrected for the Donnan equilibrium.

The following calculations were derived from three basic assumptions (7) the validity of which will be later discused:

a) During maximal water diuresis distal water reabsorption is inhibited, urine volume approaching volume of fluid delivered to the distal tubules.

b) Proximal water reabsorption is isotonic and reflects proximal solute reabsorption. Therefore, fluid reaching the more distal parts of the tubules is also isotonic.

c) Free water in the urine originates from the extraction of solute *without* water during the passage of fluid through the distal parts of the tubules. Hence, free water clearance reflects distal solute reabsorption.

Since filtered solute is given by C_FP_{OSM} (were C_F is the glomerular filtration rate in ml/min) and solute distally reabsorbed by C_{H_2O} P_{OSM},

$$(1) \qquad \frac{C_{H_2O}}{C_F} \times 100$$

defines percentage of filtered solute distally reabsorbed.

Since under maximal water diuresis urine volume approximates the volume of fluid entering the distal tubules, VP_{OSM} represents distal solute load.

$$(2) \qquad \frac{V}{C_F} \times 100$$

defines percentage of filtered solute delivered to the distal tubules, and

$$(3) \qquad \frac{C_{H_2O}}{V} \times 100$$

defines percentage of distal solute load distally reabsorbed. By substituting

$(1 - \frac{U_{OSM}}{P_{OSM}})$ V for C_{H_2O}, equation (3) may also be written

$$(4) \qquad (1 - \frac{U_{OSM}}{P_{OSM}}) \times 100$$

Since excreted solute is given by VU_{OSM}

$$(5) \qquad \frac{VU_{OSM}}{C_P P_{OSM}} \times 100$$

defines percentage of filtered solute excreted in the urine. This equation may also be written

$$(6) \qquad \frac{C_{OSM}}{C_F} \times 100$$

Results

Results are summarized in Tables I and II.

Control periods in recumbency. The glomerular filtration rate (GFR) (C_{MAN}) and the renal plasma flow (RFG) (C_{PAH}) were low in all the patients studied. Values ranged from 12.5 to 53 ml/min for the GFR and from 46.1 to 393 ml/min for the RPF. The lowest values of GFR were seen in two patients with chronic glomerulonephritis (cases 3 and 4) one of which (case 4) had also the lowest value of RPF. The highest values of both functions were seen in a patient with polycystic kidneys (case 8).

Sodium and chloride reabsorption was also low; chloride reabsorption ranged from an average of 59.2 to 95.5 % and sodium reabsorption from 64.4 to 96.2 per cent of filtered load. The highest and lowest values were seen in the same patients who evidenced the highest and lowest values of GFR (cases 8 and 4). The excretion of these ions was high; chloride excretion ranged from an average of 178 to 582 microEq/min and sodium excretion from 202 to 593 microEq/min (cases 7 and 4 respectively).

Potassium excretion ranged from 50 to 114 microEq/min. A patient with urinary obstruction secondary to prostatic hypertrophy showed the lowest value (case 7). The highest value was seen in a patient with chronic pyelonephritis (case 2). In this case as well as in cases 3, 4 and 9, potassium excretion exceeded the filtered load, again confirming potassium excretion by the tubules.

Urinary flow ranged in the "moderate water diuresis" group from 3.8 to 7 ml/min and in the "maximal water diuresis" group from 4.85 to 14.39 ml/min. In neither group a consistent relationship between urinary flow and GFR could be established.

For reasons stated under "Material and Methods", the following values were analyzed only in 6 patients studied under maximal water diuresis.

The relationship $V/C_F \times 100$ which expresses the percentage of filtered solute escaping proximal reabsorption (distal load), was normal (12%) in a patient with chronic glomerulonephritis (case 11) and high (from 19.1 to 56.5 %) in the others. Highest values were seen in a patient with chronic pyelonephritis (case 9).

Absolute solute excretion expressed by C_{OSM} was normal or high, ranging from 2.65 to 5.68 ml/min. The lowest figure was seen in a patient with one kidney removed and the other with evidence of lithiasis and urinary tract infection (case 10). The highest values were seen in a patient with polycystic kidneys (case 8). Because the GFR was low in all the patients the percentage of filtered solute excreted in the urine, expressed by the relationship $C_{OSM}/C_{MAN} \times 100$ was high ranging from 5.1 to 28.9 % of filtered load. The lowest values were observed in a patient with chronic glomerulonephritis (case 11)

TABLE I

Effect of postural changes on renal function and electrolyte excretion in chronic renal failure. Subjects under moderate water diuresis.

Case	Diagnosis	Postural Change	Time (Min)	URINE			Cl		Na		K	PLASMA		
				C_{MAN} ml/min	Vol. ml/min	C_{PAH} ml/min	exc. mEq/min	% reabs.	exc. mEq/min	% reabs.	exc. mEq/min	Cl	Na	K
1 S.Q.	Chronic Pyelonephritis	Supine	—	—	—	—	—	—	—	—	—	—	—	—
		Supine	24	14	3.08	—	212	87.7	221	88.4	57	117	144	4.2
		Tilting	63	7	1.25	—	85	86.4	88	90.8	22	117	144	4.2
		Tilting	86	9	1.32	—	94	90.4	97	92	26	115	142	4.2
		Supine	108	31.6	3.40	—	241	93.2	255	93.3	75	115	142	4
		Supine	132	21.4	3.00	—	210	90.3	225	92.1	69	114	141	4.1
2 E.E.	Chronic Pyelonephritis	Supine	21	20	5.60	78.1	336	85.6	235	92.3	108	111	145	4.2
		Supine	44	23.5	6.50	78.4	393	85.8	247	92.4	120	112	147	4.2
		Tilting	65	18.2	4.80	50.5	283	86.8	182	92.8	77	112	147	4.3
		Tilting	108	11.3	3.00	43.5	156	88.3	105	93.4	54	112	148	4.2
		Supine	131	18.7	4.30	70.5	210	90.4	146	94.4	86	112	148	4.8
		Supine	152	18.5	4.28	55	225	89.7	145	94.4	84	112	148	4.7
3 D.Ch.	Chronic Glomerulo-nephritis	Supine	15	12.7	6.4	45.6	320	76.1	320	81.0	70	100	140	4.0
		Supine	30	12.4	6.13	46.6	306	74.5	306	81.4	61	100	140	4.0
		Tilting	45	6.6	3.33	26.5	150	78.6	146	83.3	31	101	140	4.0
		Tilting	61	7.18	2.87	26.7	93	87.8	93	90.2	28	101	140	4.0
		Supine	77	13.8	5.06	64	141	89.1	162	83.1	48	102	140	4.0
		Supine	97	9.4	3.60	36.7	79	92	76	93.2	36	100	140	4.0
4 F.L.	Chronic Glomerulo-nephritis	Supine	20	12.9	7.1	84	575	60.9	596	65.3	85	108	140	3.9
		Supine	41	12.2	7.05	84.8	590	57.5	590	63.6	91	108	140	3.9
		Tilting	60	6.0	3.21	54.4	231	65.8	234	70.6	45	107	140	3.8
		Tilting	77	3.8	1.47	46.4	67	84.3	69	86.45	34	107	141	3.9
		Supine	97	10	2.30	68.6	91	91.2	94	92.9	55	107	140	3.9
		Supine	117	12.2	2.90	70	168	87.8	174	89.3	72	107	140	3.9
5 N.M.	Probable Pye-lonephritis	Supine	33	37.2	7.09	244	283	93.1	322	93.4	92	106	138	4.0
		Tilting	57	36.6	6.20	220	211	94.7	227	95.2	68	105	138	4.0
		Tilting	83	31	4.38	200	118	96.6	146	97.3	70	106	139	3.9
		Supine	112	29.6	4.68	222	121	96.4	156	95.9	61	105	139	3.9

TAB II

Effect of postural changes on renal fun and failure. Subjects max

Case	Diagnosis	Position	Time (min)	C_{MAN} ml/m	Vol. ml/m	$\frac{V}{C_{MAN}}$	URINE C_{OSM}	URINE $\frac{C_{OSM}}{C_{MAN}} \times 100$	URINE C_{H_2O} ml/m	URINE $\frac{C_{H_2O}}{C_{MAN}} \times$	$\frac{C_{H_2}}{V}$
6 L.V.	Chronic Glomerulo-Glomerulo-	Supine	17	27	4.97	18.4	3.37	12.5	1.60	5.9	3
		Supine	42	26.3	5.52	20.9	3.50	13.3	2.02	7.7	3
		Tilting	62	14.2	2.25	15.8	2.10	14.8	0.15	1.0	
		Tilting	86	14.40	1.33	9.2	1.07	7.4	0.26	1.8	1
		Supine	108	16.8	1.90	11.3	1.53	9.1	0.47	2.8	2
		Supine	130	20.7	3.18	15.3	2.40	11.6	0.78	3.8	2
F.T. 7	Obstructive Uropathy	Supine	25	15.8	4.55	28.8	3.3	19.2	1.15	7.3	2
		Supine	43	17.3	5.15	29.8	4.1	23.6	1.05	6.5	2
		Tilting	83	14.9	3.55	23.8	2.15	14.4	1	6.7	2
		Tilting	103	9.9	1.80	18.2	1.65	16.3	0.15	1.5	
		Supine	122	14.5	2.20	15.2	2.17	14.9	-0.3	-2.6	-1
		Supine	149	15.2	3.18	20.9	2.63	17.3	0.55	0.3	1
8 N.F.	Polycystic Kidneys	Supine	16	56.6	14.06	24.9	5.25	9.2	8.81	15.5	6
		Supine	27	56	15.72	28.1	6.13	11	9.59	17.1	6
		Tilting	52	58	15	25.9	7.12	12.2	7.88	13.6	5
		Tilting	75	52.2	11.86	22.7	4.29	8.2	7.57	14.5	6
		Supine	97	49.2	11.81	24	4.08	8.3	7.73	14.7	6
		Supine	118	52.5	13.80	26.3	4.72	9	9.08	17.3	6
9 G.I.	Chronic Pielone-phritis	Supine	16	15.6	9.37	60	4.9	31.4	4.47	28.6	4
		Supine	32	15.7	8.31	53	4.15	26.4	4.26	27.1	5
		Tilting	53	15.5	8.42	54.3	4	25.8	4.42	28.5	5
		Tilting	75	12.5	5.22	41.8	2.3	18.4	2.92	23.4	5
		Supine	98	11.8	4.26	36.1	1.7	14.4	2.56	21.7	6
		Supine	121	12.8	5.21	40.7	2.1	16.4	3.11	24.3	5
10 F.F.	Uunilateral nephrecto-my, Urolithiasis	Supine	13	28.3	9.38	33.2	2.9	10.2	6.5	23.0	6
		Supine	31	23.5	7.22	30.7	2.4	10.2	4.8	20.4	6
		Tilting	54	22.7	6.34	27.9	1.8	7.9	4.6	20.3	7
		Tilting	74					Samples lost			
	Chronic Pyelone-phritis	Supine	94	19.7	1.50	7.6	1.3	6.6	0.2	1.0	1
		Supine	117	27.7	1.65	6	1.4	5.0	0.2	0.7	1
11 F.H.	Chronic nephritis nephritis	Supine	22	53.0	7.00	13.3	3.11	5.9	3.89	7.3	5
		Supine	37	53.0	5.93	11.2	2.54	4.3	3.39	6.4	5
		Tilting	58	61.0	6.38	10.4	3.19	5.2	3.19	5.2	5
		Tilting	71	51.2	4.30	8.4	2.52	4.9	1.78	3.5	4
		Supine	91	43.4	3.92	9	2.16	5.1	1.76	4.5	4
		Supine	112	59.2	6.00	10.1	3.18	5.3	2.82	4.8	4

TABLE II

al function and water and solute excretion in chronic renal jects in maximal water diuresis.

$\frac{C_{H_2O}}{C_{MAN}} \times 1$	$\frac{C_{H_2O}}{V} \times 100$	C_{PAH} ml/m	Cl exc. μEq/min	Cl % reabs.	Na exc. μEq/min	Na % reabs.	K exc. μEq/min	PLASMA Cl mEq/L	PLASMA Na mEq/L	PLASMA K mEq/L
5.9	32.2	99	278	91.0	278	92.4	69	108	144	4.6
7.7	36.6	90	303	90	303	91.5	61	108	144	4.6
1.0	6.68	52	101	92.5	101	94.8	36	107	142	4.4
1.8	19.55	55	53	96.3	55	97.2	29	106	144	4.6
2.8	24.75	72	102	93.9	104	95.4	36	106	144	4.6
3.8	24.50	92	200	90.4	203	92.7	57	106	142	4.6
7.3	25.3	55	168	89.9	189	91	45	100	140	5.2
6.5	20.4	60	188	89.8	215	90.7	54	101	140	5.2
6.7	28.2	49	130	91.8	133	93.3	35	101	140	5.2
1.5	8.3	49.5	45	96.1	59	95.5	20	99	140	5.2
-2.6	-13.6	43.5	57	96.2	73	96.2	28	100	140	5.2
0.3	17.3	58.7	89	94.4	111	94.5	27	100	140	5.2
15.5	62.6	241	253	95.8	295	96.0	83	101	138	4.2
17.1	61	264	283	95.2	330	96.5	80	101	138	4.2
13.6	52	250	240	96.1	300	96	73	101.5	138	4.2
14.5	63.8	227	166	96.7	225	97.2	56	101	138	4.2
14.7	65.4	232	153	97	189	97.1	52	100.5	139	4.2
17.3	65.8	267	179	96.8	235	96.6	51	100.5	138	4.4
28.6	47.7	68.0	486	71.7	544	73.0	86	105	137	3.8
27.1	51.3	58.6	399	77.0	449	78.0	76	106	136	3.8
28.5	52.5	59.6	396	77.3	446	77.8	80	107	136	3.8
23.4	56	58.2	198	85.7	214	86.7	55	105	136	3.8
21.7	60	56.5	149	88.7	166	89.1	53	106	136	3.8
24.3	59.7	61.4	182	87.3	192	88.3	65	106	135	3.8
23.0	69.3	167	225	92.1	235	93.5	88	96	136	4.8
20.4	65.5	136	137	94.3	151	95	68	97	136	4.8
20.3	72.6	138	95	95.9	108	96.3	66	97	136	4.9
1.0	13.3	121	18	99.1	18	99.3	79	96	135	4.9
0.7	12.1	163	82	97.1	82	97.7	87	97	136	4.9
7.3	55.5	401	301	94.2	389	94.2	92	93	133	4.1
6.4	57.2	386	255	95.1	344	94.8	81	93	133	4.1
5.2	50.0	417	230	96.1	316	95.9	89	93	133	4.1
3.5	41.5	336	116	97.7	157	97.6	62	93	133	4.1
4.5	45.0	310	108	97.4	143	97.4	60	93	133	4.1
4.8	47.0	415	189	96.7	237	96.8	67	93	133	4.1

and the highest in a case of chronic pyelonephritis (case 9).

Absolute reabsorption of solute *without* water, expressed by C_{H_2O} was normal or low, ranging from 1.10 to 9.2 ml/min. The lowest values were seen in case 7 (obstructive uropathy) and the highest in case 8 (polycystic kidneys). Because of decreased GFR, the percentage of filtered solute distally reabsorbed, expressed by the relationship C_{H_2O}/C_{MAN} x 100 was normal or high in all cases. Four patients (cases 6, 7, 8 and 11) had normal values ranging from 6.3 to 16.3 % of filtrate, the lowest corresponding to a patient with chronic glomerulophritis (case 6) and the highest to a patient with polycystic kidneys (case 8). The two remaining patients, both with chronic pyelonephritis, showed very high values, 27.8 and 21.3 % of filtrate.

The distal solute load distally reabsorbed, expressed by the relationship C_{H_2O}/V x 100 was low in all the patients studied, ranging from 22.8 to 67.4 %. The lowest value was seen in case 7 (obstructive uropathy) and the highest in case 10 (chronic pyelonephritis in a single kidney).

Maximal and minimal values in seven normal subjects and in six patients with chronic renal failure under maximal water diuresis are compared in Table III. Normal values have been recalculated according to the data given by Sursthin and White (7) from experiments conducted under conditions similar to ours. Normal values between brackets have been taken from H. Smith. These same values are graphically represented in figure 1.

Postural changes. When patients were placed in the semi-vertical position, all of them showed a reduction in GFR (C_{MAN}), RPF (C_{PAH}) and urinary flow as well as in chloride, sodium, potassium, solute (C_{OSM}) and solute free water (C_{H_2O}) excretion (Fig. 2).

RPF (C_{PAH}) decreased to values ranging from 57 to 89.3 % (cases 3 and 9) and GFR (C_{MAN}) to values from 31 to 87.4 % (cases 4 and 8) of average control values in recumbency. In the two cases in which percent fall was maximal, RPF and GFR reached minimal absolute values, 26.7 and 3.8 ml/min respectively.

Chloride excretion decreased to values ranging from 10 to 57 % (cases 10 and 8), sodium excretion to values ranging from 9.3 to 60.3 (cases 10 y 8) and potassium excretion to values ranging from 38.7 to 61 % (cases 4 and 11) of control levels in recumbency.

Urine volume decreased to values ranging from 20.7 to 82.4 % (cases 8 and 4), solute excretion (C_{OSM}) to values from 31.2 to 76.6 % (cases 6 and 11) and solute free water excretion (C_{H_2O}) to values ranging from — 26 to 83.8 % (cases 7 and 8) of control values in recumbency. The first figure corresponds to a patient in whom the C_{H_2O} became slightly negative reflecting reabsorption of solute-free water during maximal water diuresis.

Fall in urine flow and in chloride, sodium, solute (C_{OSM}) and solute-free water (C_{H_2O}) excretion exceeded fall in GFR, chloride reabsorption increasing from 1.5 to 32 % and sodium reabsorption from 1.0 to 28.5 % of filtered load (Fig. 3). Maximal increase in percent sodium and chloride reabsorption was seen in the patient with lowest initial value (case 4) and minimal increase in the patient with the highest initial value (case 8).

In all cases the relationships V/C_{MAN} x 100, C_{OSM}/C_{MAN} x 100 and C_{H_2O}/C_{MAN} x 100 also decreased. Fall in C_{H_2O} exceeded fall urine volume in all cases except in case 9 in whom the relationship C_{H_2O}/V x 100 increased from 49.5 to 60 %. The significance of these findings will be discussed later.

Recovery. In the final periods in recumbency all the values tended to return to control levels although these

TABLE III

Comparative values of the equations in normal subjects and in patients with chronic renal failure (*)

EQUATION	MEANING	VALUES	
		Normals	Chronic renal disease
$\frac{V}{C_F} \times 100$	Percentage of filtered solute load delivered to the distal tubules.	11.3 to 18.4 %	12.2 to 56.5 %
$\frac{C_{H_2O}}{C_F} \times 100$	Percentage of filtered solute load, distally reabsorbed.	7.59 to 13.6 % 7 to 16 % (H. Smith)	6.8 to 27.8 %
$\frac{C_{H_2O}}{V} \times 100$	Percentage of distal solute load, distally reabsorbed.	71 to 89 %	22.8 to 67.4 %
$\frac{C_{OSM}}{C_F} \times 100$	Percentage of filtered solute load that is excreted.	2.37 to 3.88 % 1 to 3 % (H. Smith)	5.1 to 21.4 %

(*) Normal values were recalculated from the data given by Surtshin and White (7) and by H. Smith (6).

TABLE IV

CASE 8. — *Values of the equations, uncorrected and corrected for a hypothetical constant* $T^c_{H_2O}$ *of 4 % of filtrate. Figures in the left column are the average of two initial periods in recumbency. Figures in the right column correspond to the second period in the semivertical position.*

ml/min.	Supine	Tilting	Difference
C_{MAN}	16	9.9	+ 6.1
C_{H_2O}	1.10	0.15	+ 0.95
V	4.85	1.80	+ 3.05
$T^c_{H_2O}$	0.64	0.64	0
$\frac{C_{H_2O}}{C_{MAN}} \times 100$	6.9	1.5	+ 5.4
$\frac{C_{H_2O} + T^c_{H_2O}}{C_{MAN}} \times 100$	10.8	7.9	+ 2.9
$\frac{C_{H_2O}}{V} \times 100$	22.3	8.3	+ 14.0
$\frac{C_{H_2O} + T^c_{H_2O}}{V + T^c_{H_2O}} \times 100$	31.7	32.3	− 0.6

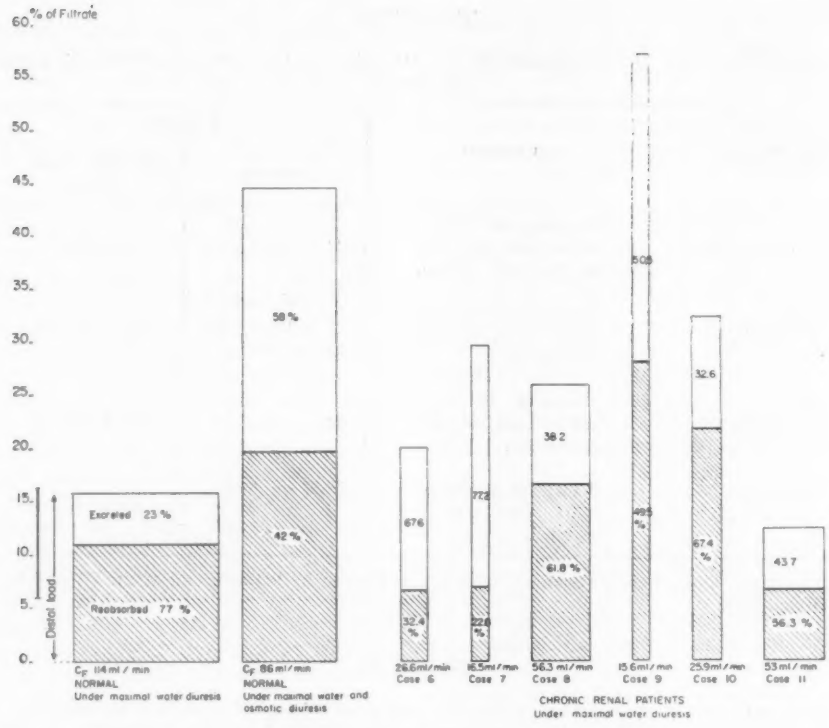

FIG. 1. — *Solute reabsorption and excretion during maximal water diuresis in:*

a) Normal Subjetc.

b) Normal Subjetc under osmotic diuresis.

c) Patients with chronic renal failure.

Figures used in the construction of graph a) are the average values of seven normal subjects studied by Surtshin and White (7) under conditions similar to ours and have been recalculated from the data given by these authors. Figures used in the construction of graph b) are maximal values of a patient with diabetes insipidus placed under mannitol diuresis and have been recalculated from the data given by de Wardener and del Greco (12). Figures used in the construction of graphs c) are the average of two initial periods in recumbency. Heights represent values expressed as percentages of filtrate. Width of columns correspond to glomerular filtration rate expressed as ml per minute. Total areas represent absolute values expressed in ml/min. Figures within columns refer to percentages of distal load. Full line on vertical scale indicate normal range of C_{H_2O} per 100 ml of filtrate.

were seldom attained. Generally speaking, recovery was more clearcut during the last period in recumbency since in the first the effect of preceding tilting still persisted.

Recovery started earlier and was complete or more nearly complete for RPF and GFR. RPF reached or exceeded control levels in cases 6 to 11. The same was observed for GFR in cases 1, 3, 4, 10 and 11. Recovery of the other functions was slower and less complete. For

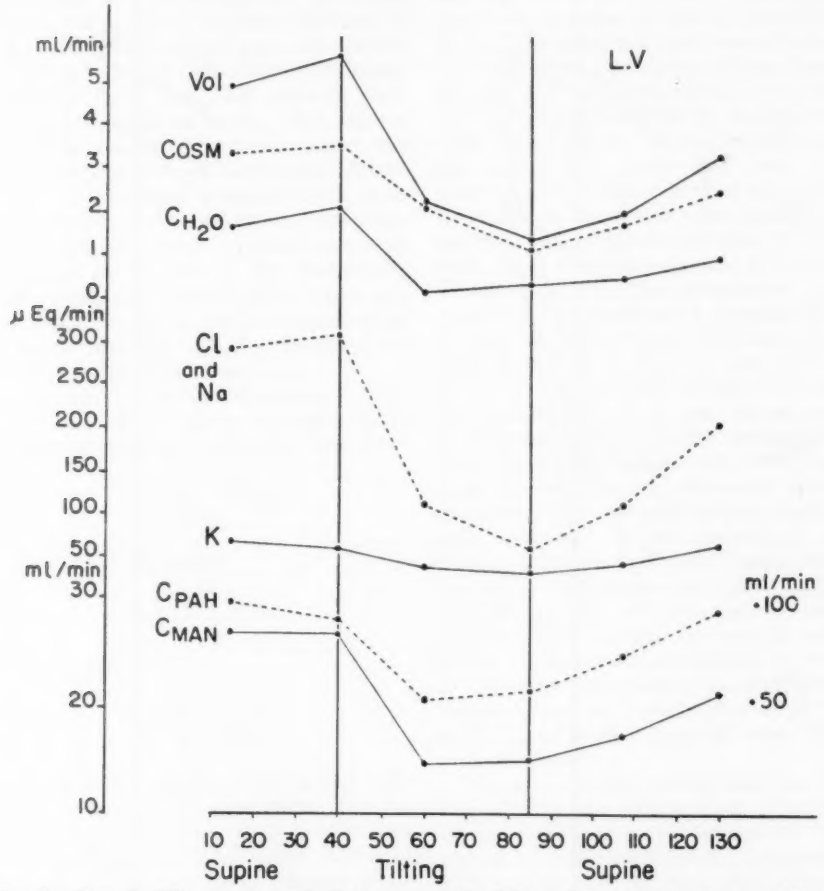

FIG. 2.—CASE 6. *Effect of postural changes on GFR, RPF and water and solute excretion Absolute values.*

instance, the percentage of reabsorbed sodium and chloride, though lower than in the semi-vertical position, was still higher than in the control periods even when GFR had already returned to, or gone beyond initial values. Likewise, the relationships V/C_{MAN} x 100 and C_{H_2O}/C_{MAN} x 100 returned to initial values only in case 8 and the relationship C_{OSM}/C_{MAN} x 100 only in cases 8 and 11.

DISCUSSION

Validity of calculations. Assumptions b) and c) have received strong support from experimental work, specially so from micropuncture studies which need not be considered here. An excellent discussion of the subject may be read in the paper by Berliner et al. ([8]). On the contrary, the assumption a) that under maximal water diuresis, distal water

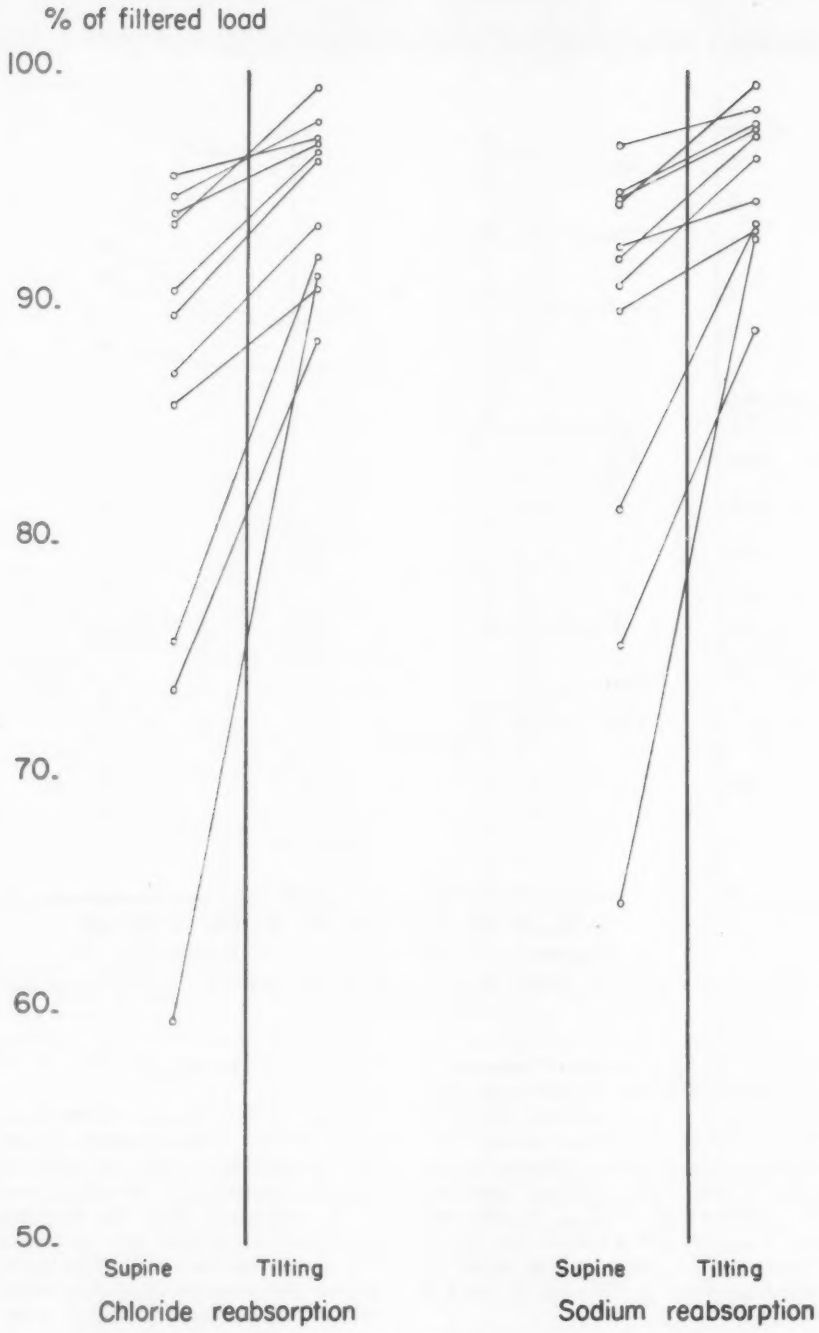

FIG 3.—*Effect of postural changes on sodium and chloride reabsorption. Values on the left are the average of two initial periods in recumbency. Values on the right are the minimal ones found during the semivertical position or the first subsequent period in recumbency.*

reabsorption is completely inhibited may not be quite correct. Berliner (9) obtained urine hypertonic to plasma during maximal water diuresis by markedly reducing the GFR through constriction of a renal artery. This was explained assuming that even under maximal water diuresis some water diffuses back through the collector ducts independently of the antidiuretic hormone. When distal solute load was much decreased by the fall in the glomerular filtration rate, distal solute extraction was so low that the water reabsorbed by the collector ducts slightly exceeded the free water of the urine.

If some water is reabsorbed by the collector ducts under maximal water diuresis, equation 1 should be rewritten:

$$(1') \qquad \frac{C_{H_2O} + T^c_{H_2O}}{C_F}$$

equation 2 becoming

$$(2) \qquad \frac{V + T^c_{H_2O}}{C_F}$$

and equation 3,

$$(3') \qquad \frac{C_{H_2O} + T^c_{H_2O}}{V + T^c_{H_2O}}$$

where $T^c_{H_2O}$ represents the water reabsorbed by the collector ducts. The introduction of $T^c_{H_2O}$ in the equatinos results in higher final values. Unfortunately the percentage of filtered water reabsorbed by the collector ducts as solute-free water has not been measured. Nevertheless, it most probably does not exceed the percentage reabsorbed under osmotic diuresis during the hydropenic state. This value has been shown not to significantly differ in patients with chronic renal failure of moderate degree and in normal subjetes (1). Therefore, we may conclude that even though the uncorrected equations may not be absolutely exact, they are still valid for comparative purposes in face of unchanging GFR. This is not the case when there is a pronounced fall in the GFR. In fact, if during maximal water diuresis some solute-free water diffuses back through the collecting ducts at a constant rate independently of changes in GFR, this constant amount of reabsorbed solute-free water will exert a uneven influence on the final urine volume and on solute-free water excretion derived from different volumes of filtrate. This becomes apparent in Table IV which presents the uncorrected and corrected equations for a hypothetical $T^c_{H_2O}$ of 4 % of filtrate. The data pertains to case 8. As may be seen, had a $T^c_{H_2O}$ of 4 % of really existed filtrate, the *actual* decrease in the percentage of filtered solute distally reabsorbed (C_{H_2O}/C_F x 100) would have been less than the one shown by the uncorrected equation and no *actual* decrease would have occurred in the percentage of distal solute load distally reabsorbed (C_{H_2O}/V x 100). Related to this, it is significant that in the only case of the seven normals studied by Surtshin and White (7) in conditions similar to ours in whom the relationship C_{H_2O}/V x 100 dropped from an initial value of 89 % to 76 %, GFR also dropped from 89.9 to 75.9 ml/min.

The absolute and relative decrease in C_{H_2O} during the semivertical position in cur cases might also be due to the release of some antidiuretic hormone. This can be inferred from the investigations of Brun et al. (10) and of Pearce and Newman (11). Brun et al. observed that the blood of patients placed in a semivertical position and with clinical signs of collapse, when transfused to normal subjects, elicited in the latter a significant antidiuretic response. Pearce and Newman found that the oliguria of standing could be inhibited by alcohol, a known inhibitor of ADH secretion.

In conclusion, even though the equations used in this study may not be quite correct, they are at least valid for

comparative purposes when used in the basal equilibrated conditions of the control periods in recumbency. However, any interpretation of the fall in these values during the semivertical position must take into account the possible influence of the drop in the GFR or the existence of some antidiuretic activity.

Interpretation of results. According to what has been previously said, low values of $C_{H_2O}/V \times 100$ in patients with chronic renal failure under maximal water diuresis placed in basal conditions should be considered as an evidence of low percentage of distal solute load distally reabsorbed. This might be due to: 1) an *absolute* low distal solute reabsorption reflecting a true inability of the kidneys to dilute the urine or 2) a *relative* low distal solute reabsorption secondary to an increased distal load.

In normal subjects, increase in distal load as seen in osmotic diuresis brought out by urea or mannitol is followed by decrease in the percentage of distal solute load distally reabsorbed. Recalculation of de Wardener and del Greco's data from a patient with diabetes insipidus ([12]) shows that the distal solute load increased during mannitol infusion from 10 to 46 % of filtrate, distal solute reabsorption simultaneously decreasing from 76 to 42 % of distal load. Osmotic diuresis was evident in all of our patients with chronic renal failure, judging from high urine volume coupled to low percent sodium and chloride reabsorption and high percent excretion of filtered solute (5 to 29 %). Normal values: 1 to 3 % of filtrate according to H. Smith et al. ([6]) and 2.37 to 3.88 % according to Surtshin and White's data ([7]). Therefore, patients with chronic renal failure under water diuresis might be likened to normal subjects under combined water and osmotic diuresis.

However, only in cases 8, 9 and 10 could the increase in distal load characteristic of osmotic diuresis brought out by urea or mannitol, account for the low percentage of distal solute load distally reabsorbed. In these three cases the distal load was 26.5, 56.5 and 32 % of filtrate, values considerably higher than those found in normal subjects under maximal water diuresis and closer to values seen in normal subjects under combined osmotic and water diuresis. On the contrary, in cases 6 and 11, the distal load was well within normal limits (19.6 and 12.2 % of filtrate); in spite of this, the percentage of distal solute load distally reabsorbed was lower than normal (34 and 56 %). In cases 7 and 8 distal load was about the same (29.3 and 26.5 % of filtrate); however, percentage of distal solute load distally reabsorbed was in case 7 much lower than in case 8 (23 and 62 %). It looks as if in cases 6, 7 and 11 osmotic diuresis were the effect rather than the cause of the low percentage of distal solute load distally reabsorbed.

If high distal load were the only factor playing in the low percentage of distal solute load distally reabsorbed it could be expected that a reduction in distal load would increase the percentage distally reabsorbed. In all our patients the distal load decreased during the semi-vertical position as a consequence of the fall in GFR. However, in only one instance (case 9), did the relationship $C_{H_2O}/ \times 100$ increase, while in the other cases it decreased – slightly in cases 8 and 11 and markedly in cases 6, 7 and 10. Truly, decrease in the ratio during the semivertical position cannot be taken as an evidence of lower percentage of distal solute load distally reabsorbed. As has been stated before, it may merely be an effect of reduced GFR or of some antidiuretic activity.

The presence of some antidiuretic activity during the semi-vertical position, independent of osmolality and related to volume changes may be presumed in cases 6, 7 and 10 by the persistence

of decreased C_{H_2O} during the subsequent period in recumbency when the GFR had already returned to previous values. In case 10 this assumption is further supported by the occurrence of moderate collapse similar to that described by Brun et al. (10). The antidiuretic activity seems less likely in the remaining cases who did not show any circulatory disturbance. Moreover our patients, unlike those of Pearce and Newman (11) and Brun et al. (10), were under maximal water diuresis. However, though decrease in the relationship $C_{H_2O}/V \times 100$) during the semivertical position cannot be taken as an evidence of low percentage of distal solute load distally reabsorbed, it seems rather unlikely that an *actual* increase of this value had occurred in most cases under these circumstances.

High distal solute load, as seen in chronic renal failure, may possibly be due to the increased filtered load secondary to the reduction in the nephron population. The increase during the semi-vertical position in the percentage of sodium and chloride reabsorbed, from low control figures to figures approaching normality, could be explained in our experiments by the decrease in the filtered load provoked by the fall in the GFR. However, the fact that this percentage remained high during the final periods in recumbency when the GFR had already returned to previous levels, reveals a good capacity of the proximal tubules to increase solute reabsorption. On the other hand, the persistence of the effects of postural change on sodium and chloride reabsorption during the subsequent periods in recumbency, suggests a humoral mechanism.

The percentage of filtered solute distally reabsorbed, expressed by the relationship $C_{H_2O}/C_F \times 100$ was normal (6.5 to 16.3 %) in four cases of chronic renal failure and high (27.8 and 21.5 %) in two others. Very likely, osmotic diuresis invoked as a cause for the inability of the diseased kidneys to dilute the urine was also responsible for the high values of the ratio found in cases 9 and 10, since in these cases the distal solute load was also very high (56.6 and 31.9 per cent of filtrate). Rosenbaum et al. (14) found that the diuretic response to water increases with previous solute loading. This has been explained by Wesson and Anslow (15), H. Smith (16) and Ladd (17) by the addition of unreabsorbed isosmotic fluid to the urine volume, C_{H_2O} keeping constant during the peak of water diuresis. However although in Wesson and Anslow's (18) urea loading experiments C_{H_2O} did not significantly change in absolute values, as the GFR steadily decreased, C_{H_2O} increased in terms of percentage of filtration from 12 to 20 %. Relman et al. (19) observed that mannitol infused during maximal water diuresis increased sodium and chloride excretion but decreased their concentration in the urine thus suggesting an increase in free water excretion. This assumption was confirmed by de Wardener and del Greco (12) by direct measurement of C_{H_2O} under similar conditions. Also Kleeman et al. (20) were able to increase C_{H_2O} by increasing solute excretion through previously feeding a high protein – high salt diet. Finally, Orloff and Walser (21) concluded from their experiments carried out in a case of pitressin-resistant diabetes insipidus, that the rate of free water excretion increases *pari passu* with solute excretion. In experimentally produced unilateral nephritis, Bricker et al. (12) found a slightly higher C_{H_2O} per 100 ml of filtrate in the damaged kidney than in the normal one, but the difference observed was rather small. This might be explained by the presence of a normal contralateral kidney which assures a nephron population of more than 50 %. On the other hand, the fact that free water excretion increases with solute excretion makes

doubtful that normal values of C_{H_2O} per 100 ml of filtrate could be taken as an evidence of integrity of the dilution mechanism, high distal solute load apparently being able to compensate for low distal solute reabsorption.

In conclusion, osmotic diuresis is most probably the main factor playing in normal or high values of C_{H_2O} per 100 ml of filtrate in patients with chronic renal failure. However, this cannot be taken as a true index of integrity of the dilution mechanism. Moreover, it seems rather unlikely that osmotic diuresis is the only factor playing in the inability of the damaged kidney to dilute the urine.

Summary

Eleven patients with chronic renal failure in the polyuric phase were studied under maximal and moderate water diuresis. All patients were in a state of osmotic diuresis evidenced by high distal solute load and/or high percentage of filtered solute excreted in the urine. The percentage of distal solute load distally reabsorbed was low compared to normals, but because it operated over higher distal solute loads, the percentage of filtered solute distally reabsorbed was normal or high. For this reason, it is believed that normal or high values of C_{H_2O} per 100 ml of filtrate in chronic renal failure do not necessarily imply integrity of the dilution mechanism, high distal solute load apparently being able to compensate for low distal solute extraction. On the other hand, low percentage of distal solute load distally reabsorbed cannot always be explained on the basis of high distal load.

Change to the semi-vertical position elicited in all the studied patients a moderate to marked drop in GRF. The percentage of reabsorbed water, chloride and sodium approached normal values. C_{H_2O} and C_{OSM} dropped significantly, the former even to the point of negativity. The significance of these findings are discussed.

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THYROID GLAND AND HYPERTENSION *

JOSÉ A. ZADUNAISKY **
(Instituto de Fisiología, Facultad de Ciencias Médicas, Buenos Aires.)

SEVERAL years ago, Braun Menéndez (1) advanced a working hypothesis which postulated that kidney size might be controlled by some specific renotrophic substances and that a failure of the kidneys to increase their size in response to an increase in the amount of these circulating renotrophic substances would lead to the hypertensive state. Such an increase in renotrophic substances could be induced either by extrarenal factors or by a reduction of the renal mass.

Indeed, when several substances with proven ability to increase renal size were studied for their effect on the blood pressure of rats with a mild renal experimental hypertension, the levels of hypertension were always aggravated (1-6).

Although previous work had not shown an appreciable effect of thyroid hormone on hypertension in dogs (7-9), nevertheless, because of the known influence of the thyroid on renal size, Braun Menéndez also investigated its effect on the blood pressure of rats with renal hypertension. Not only did administration of thyroid hormone increase the blood pressure, but also thyroidectomy was followed by regression of the hypertension (3-5). The reason for the difference in the response of the two species, rat and dog is not clear.

As a further extension of the above work, the present experiments were designed to evaluate: 1) the influence of thyroid hormone on blood pressure, renal growth, size and function; 2) the relationship between the modifications in renal size and renal function in euthyroidism, hyperthyroidism and hypothyroidism.

METHODS

Male and female white rats, weighing from 150 to 300 grams were used. All the animals were kept on the standard diet of the Institute.

Blood pressure was determined on the tail of the rats by the microphonic method of Freedman and Free (10) dur-

(*) This work was done under the direction of late professor Eduardo Braun Menéndez and presented as a Thesis for the degree of Doctor in Medicine to the University of Buenos Aires.

(**)Fellow of the Santamarina Foundation during part of this work, and from the "Consejo Nacional de Investigaciones Científicas y Técnicas", Argentina. Present address: Institute of Biological Chemistry, University of Copenhagen, Denmark.

ing light ether anesthesia. Each animal's blood pressure is the mean of three determinations taken in the fasting state. These mean blood pressures were then averaged to give a daily value for the whole group. The values obtained by this method in this laboratory from a large number of normal rats have ranged from 80 to 130 mm Hg. Accordingly, only a blood pressure of 140 mm Hg or higher is accepted as indicating hypertension. *Renal hypertension* was produced by placing an 8-shaped ligature on one kidney, with the removal of the contralateral kidney one week later. With this technique there is always a latent period of about one month before hypertension appears (11).

Renal weights were determined immediately after sacrificing the rats. The kidneys were removed, blotted on filter paper and weighed in less than 5 minutes. Values are expressed as mg per 100 square cm of body surface which was calculated by the formula $11.36 \times W^{0.66}$.

Radiothyroidectomy was produced by the intraperitoneal injection of 1 millicurie of I^{131} in a volume of 1 ml. This dose is sufficient to produce a complete destruction of the thyroid gland with sclerosis in 30 days (12).

I^{131} trapping by the thyroid was determined in the intact, anesthetized rat 24 hours after the intraperitoneal injection of 1 microcurie of radioiodine. The maximal count in the thyroid region was determined by a scintillation counter provided with a conical colimator. Control blood pressure was determined before the initial injection of radioiodine.

Injections of 3-5-3' triiodothyronine (T3) were given subcutaneously in daily dosis varying from 2 μg to 100 μg. In some of the experiments, thyroid powder 30 mg per day per rat, was administered orally.

Renal clearances, glomerular filtration rate (G. F. R.) and renal plasma flow (R. P. F.) were determined by the method of Braun Menéndez and Chiodi (13), except on a few occasions when the method of Binet et al. (14) was used. Both methods are based on a single subcutaneous or intraperitoneal injection of inulin and either diodrast or p-amino hippurate and the collection of only one sample of blood at the end of the clearance period.

Results

Effect of T3 on blood pressure: To one of two groups of 5 rats each in which renal mass had been reduced by unilateral nephrectomy and an 8-shaped ligature placed on the remaining kidney, 50 μg of T3 per day and per rat were given immediately after the second operation, for a period of 17 days. Blood pressure rose after one week of treatment (Fig. 1), and remained at hypertensive levels during the days of treatment. Nine days after discontinuing the T3, blood pressure had returned to normotensive control values. All of the second group of similarly operated rats who had not received T3 developed hypertension after approximately one month of post-operative normotension. A third group of 7 unoperated rats, but given also 50 μg of T3 showed no changes in blood pressure.

Effect of T3 on unilateral nephrectomized rats: One of two groups of rats (17 treated and 10 controls) in which renal mass had been reduced 50 % by unilateral nephrectomy (the excised kidney was weighed) was given 100 μg of T3 for 5 days, beginning the day prior to the operation. At the end of this period 7 rats of this group and 5 from the control group were sacrificed and the remaining kidney excised and weighed. On the 14th day of the experiment the rest of the rats of the two groups were sacrificed and their remaining kidney weighed.

The kidneys removed from both

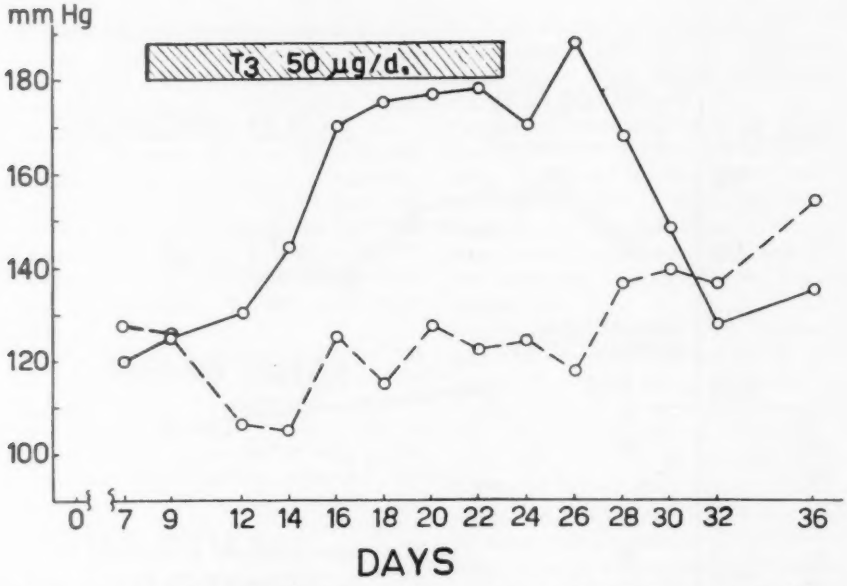

FIG. 1. — *Effect of T3 on the blood pressure of rats with an 8-shaped ligature of one kidney and removal of the contralateral one. T3 was administered to the group represented by the unbroken line immediately after the second of these two operations. The dotted line represents similarly operated, non-treated controls.*

groups of rats after 5 days of treatment and 4 days of compensatory hypertrophy were hypertrophied (Fig. 2), when compared with the kidneys removed in performing the unilateral nephrectomy. Albeit, the remaining kidney from the unilaterally nephrectomized rats after T3 treatment weighed 20.5 % more ($P < 0.01$) than the remaining hypertrophied kidneys from the untreated rats.

The rest of the two groups of rats sacrificed 13 days after unilateral nephrectomy and 9 days after one of them had received T3 treatment, showed no difference in the size of the remaining hypertrophied kidney when their weights were compared.

Blood pressure, determined daily during T3 treatment and every two days until the end of the experiment, was never greater than normotensive control values. Thus, it is possible with T3 to superimpose an additional degree of hypertrophy on that which normally follows a reduction in renal mass. Further, following cessation of T3 administration there is a rapid and total regression in 9 days of the superimposed hypertrophy to the level achieved by the kidneys of the unilaterally nephrectomized not-treated rats.

The only difference between this experiment and the one previously described is, in fact, the existence of an 8-shaped ligature on the remaining kidney after unilateral nephrectomy. We will return to this point in the discussion.

Effect of T3 on the renal weight of normal and hypothyroid rats: The administration of T3 to normal rats pro-

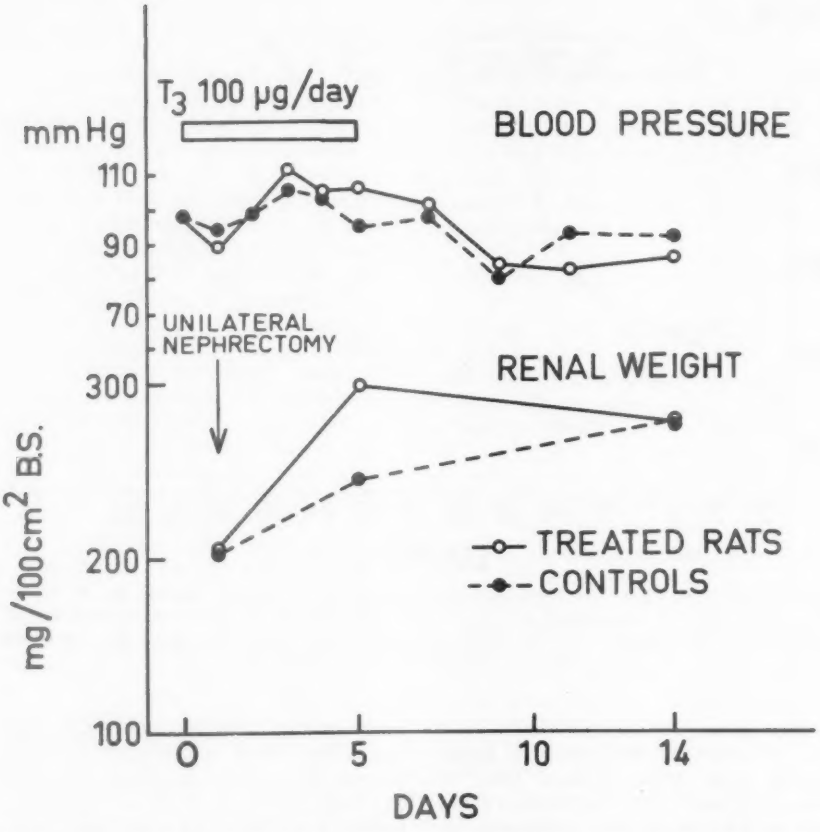

FIG. 2. — *Effect of T_3 on the blood pressure and the renal size of the remaining kidney after unilateral nephrectomy. Injections of T_3 were started one day before the operation and continued until the 5th day of experiment when part of both treated and controls were sacrificed and their kidneys weighed. From then on the values correspond to the rest of the animals left.*

duced a hypertrophy of both kidneys as the treatment with thyroid extracts or thyroxine also does ([15, 16]). On the basis of this observation an experiment was done in 4 groups of 10 rats each. One group was left as control and the others were treated during 10 days with 40 μg of T3 per day and per rat to promote the hypertrophy of the kidneys. After this period, one of the 3 groups receiving T3 was killed, and the other two were left alive during 10 and 20 more days after discontinuing the injections. The hypertrophy of the kidneys (Fig. 3) was 30 % over the control values ($P < 0.01$) and 20 days after the end of the treatment the kidneys of the last group showed no difference with the controls. Ten days after the end of the injections of T3, the weight of the

kidneys was still over the normal values.

The hypertrophic effect of T3 was also tested in radiothyroidectomized rats which showed a reduction in renal size.

Different small doses of T3 were administered to 3 groups of 3 radiothyroidectomized rats during 10 days and at the end of the treatment the rats were killed and the kidneys weighed. There was an hypertrophic effect which was proportional to the doses used (Fig. 3).

Thyroid gland and renal clearances: G. F.R. and R. P. F. were examined in a large number of euthyroid, thyroid-treated and hypothyroid rats. Table I shows the general results for inulin clearance and diodrast clearance for controls, thyroid-treated and radiothyroidectomized rats. The administration of 30 mg per day of thyroid powder to normal rats did not produce any increase in functions, nevertheless 50 μg of T3 per day, during 10 days produced a definite increase of both G. F. R. and R. P. F. Radiothyroidectomy produced a remarkable reduction in both functions. When these radiothyroidectomized rats were treated with thyroid powder, at the same dose as the normal rats, or with 20 μg per day of T3, the renal functions were restored to normal values (Table II). In these radiothyroidectomized rats the renal clearances were determined twice, before and after the replacing therapy with thyroid powder or T3.

Correlation between renal clearances and renal weights: Values for G. F. R. and R. P. F. from hypo, euthyroid and thyroid-treated rats were plotted against the respective weights of both kidneys from the same rat. At the end of the clearance period part of the different groups of rats included in Table I, 68 in total, were immediately killed and their kidneys weighed. A good statistical correlation ($P < 0.01$) between renal clearance and the different renal weights which accompanied the three

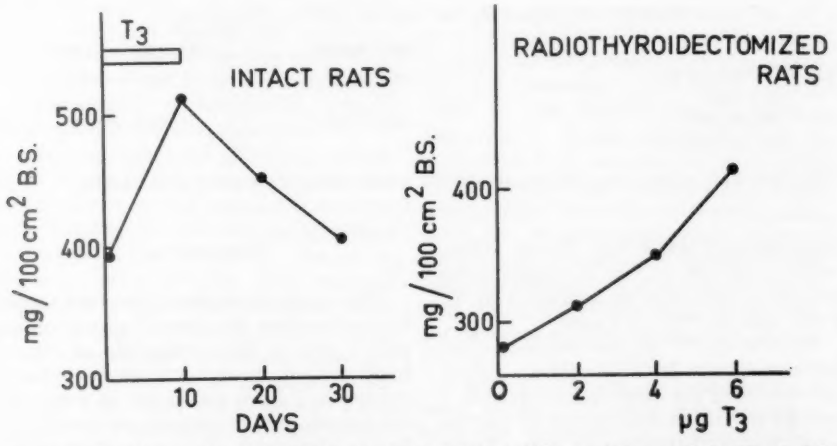

FIG. 3.—*Effect of T3 administration on the weight of both kidneys of the rat.* Left: *The points represent the mean renal weights for groups of 10 normal rats treated with T3 and then sacrificed at different times after the discontinuation of the treatment.* Right: *Each point represents here the mean renal weights for groups of hypothyroid rats treated with the different doses of T3 during 10 days.*

TABLE I

Glomerular filtration rate (G. F. R.) and Renal plasma flow (R. P. F.) in normal, radiothyroidectomized and thyroid treated-rats

	G. F. R.	R. P. F.	P
NORMALS	0.285 ± 0.014 (46)	0.98 ± 0.08 (31)	
THYROID POWDER TREATED	0.312 ± 0.014 (35)	1.06 ± 0.08 (28)	< 0.1
T3 TREATED	0.552 ± 0.079 (8)	1.54 ± 0.23 (8)	< 0.01
RADIOTHYROIDECTOMIZED	0.190 ± 0.007 (51)	0.32 ± 0.02 (33)	< 0.01

The values are expressed as ml per minute per 100 square centimeters of body surface and followed by ± the standard error of the mean. Figures in brackets represent number of animals. P values are those found between the normal rats and each of the other groups.

TABLE II

Effect of thyroid treatment on renal function of radiothyroidectomized rats

	G. F. R.		R. P. F.	
	Before	After	Before	After
THYROID POWDER TREATED	0.138 ± 0.02	0.275 ± 0.03	0.26 ± 0.03	0.75 ± 0.09
T3	0.195 ± 0.05	0.319 ± 0.08	—	—

The values are expressed as ml per minute per 100 square centimeter of body surface, followed by ± the standard error of the mean for two groups of 10 radiothyroidectomized rats. Thyroid powder was administered orally during 15 days and T3 (20 μg) during 10 days. The increases in G. F. R. or R. P. F. were statistically significant ($P < 0.01$). (See text.)

TABLE III

Radioiodine trapping by the thyroid gland in hypertension

	CONTROLS	HYPERTENSIVE
BLOOD PRESSURE (mm Hg)	119 ± 3.4	182 ± 8.1
I 131 TRAPPING (% after 24 hours)	24.0 ± 3.0	26.6 ± 3.0

The figures are mean values for two groups of 6 rats, followed by ± error of the mean.

experimental states was found. Thus, with a change in size a proportional change in function as measured by G. F. R. and R. P. F. is found.

Uptake of I^{131} by the thyroid in hypertensive rats: In Table III are shown the results of the determinations of I^{131} uptake by the thyroid gland of normal and chronic hypertensive rats. There was no difference in the percentage of radioiodine trapped by the gland between the hypertensive and control rats, after 24 hours.

Discussion

The experiments here described tend to confirm that the thyroid gland could play a role in the mechanism of renal hypertension in the rat ([3, 5]). When approached from the point of view of the renotrophic hypothesis advanced by Braun Menéndez, the results show that a link between renal growth and renal hypertension is very likely. The only case in which triiodothyronine produced hypertension was in the presence of

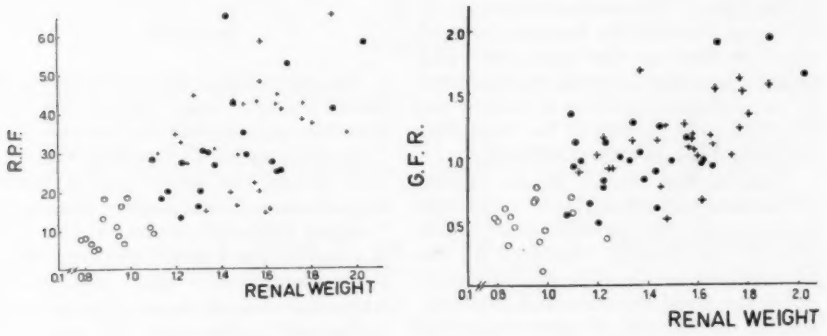

FIG. 4. — *Correlation between renal function and renal weights: Radiothyroidectomized rats; o Normal rats; + Thyroid treated rats. G. F. R. and R. P. F. are expressed in ml per minute and the weights of both kidneys in grams.*

a 50 % reduction of the renal mass and an 8-shaped ligature of the remaining kidney. The degree of hypertension was very severe and it appeared during the silent period before hypertension develops when this technique, described by Grollman (11), is used. Unilateral nephrectomy alone, in the absence of the 8-shaped ligature of the remaining kidney does not sensitize the rat to the hypertensive effects of T3, but a superimposed growth of the remaining, compensatorily hypertrophied, kidney accompanies its administration.

The time taken for the blood pressure to return to control values after discontinuation of the treatment and the time required for the remaining kidney to return to the weight of controls is the same, about 10 days. The evidence suggests that the similar behaviour is induced through factors other than chance. If one reasons according with the renotrophic idea, it is possible to say that in both cases the effect of T3 is due to its renotrophic properties and that the restoration to normal of both parameters, renal size and blood pressure, requires the same period of time which is required for the increased circulating renotrophic substances to return to normal.

Further evidence for this hypothesis is afforded by the experiment of Ogawa and Nowinsky (17) in which they showed that the addition of plasma from unilateral nephrectomized rats to tissue culture of renal cells resulted in a greater number of mitosis in the cultured tissue. Thus, it is possible that a humoral factor present during compensatory hyperthophy of the kidney might well be the hypertensive substance suggested by Braun Menéndez (1).

As regards to the increase or decrease of glomerular filtration rate and renal plasma flow that follows thyroid treatment or radiothyroidectomy respectively, or the restoration to normal of the lower renal function of the hypothyroid rats, the results demonstrate that the rat behaves as the dog in this respect (18-21).

The linear correlation found between renal weight and renal function when both are decreased by radiothyroidectomy or increased by thyroid treatment shows that these parameters are interdependent. However, it can not be said from these experiments whether the size

of the kidney determines its function or viceversa. Possibly the increases in renal function due to the treatment with thyroid hormones would be hampered by an 8-shaped ligature as well as the possibilities of growth of the renal mass are hampered in this condition.

The trapping of I^{131} by the thyroid gland in hypertensive rats is unchanged from normal. One would expect certain changes in thyroid function in the hypertensive rat since thyroidectomy reduces the renal experimental hypertension of the rat. On the other hand this was only one of the many tests for thyroid function. In fact, Brull and Mechei [22] have studied thyroid function in hypertensive dogs and concluded that there was an increase in the release of thyroid hormones during the development of hypertension due to the application of a Goldblatt clamp to the renal artery.

Finally, the observed increases in renal weight of normal and hypothyroid rats with the administration of thyroid hormone are in agreement with previous literature [15, 16] but the effects were much more marked than prior observations due to potency of triiodothyronine and the doses used. The regression of the renal weights to normal values after discontinuation of the treatment was rapid, 20 days.

From previous publications, and from the experiments here described, we may conclude that the thyroid gland plays a role in renal experimental hypertension of the rat as Braun Menéndez has pointed out and that, possibly, the mechanism of its effect is indirectly mediated through "renotrophin" or renotrophic substances which are increased during thyroid hyperfunction, treatment with thyroid hormones and renal experimental hypertension.

Summary

The influence of thyroid hormone on blood pressure, renal growth, size and function were studied in the white rat. Triiodothyronine (T3) administered to rats during the silent period before hypertension develops, after placing an 8-shaped ligature on one kidney and removal of the contralateral, produced a hypertensive state which disappeared when the treatment was discontinued. Unilateral nephrectomy did not sensitize the rats to the hypertensive effects of T3, but produced a superimposed hypertrophy of the remaining kidney. This superimposed hypertrophy disappeared rapidly and the treated kidney regressed to the same size achieved by kidneys of untreated unilaterally nephrectomized controls. The test of radioiodine uptake by the thyroid gland after 24 hours, performed in hypertensive and in normotensive control rats showed no appreciable differences.

T3 administration also produced hypertrophy of the kidneys of normal rats and rapid regression to control values after discontinuation of the treatment. Radiothyroidectomized rats showed an increase in kidney weight proportional to the doses of T3. G. F. R. and R. P. F. values were reduced in radiothyroidectomized and augmented in thyroid treated rats. A good statistical correlation was found between the modifications of renal function and those of the renal weights in hypothyroidism, euthyroidism and hyperthyroidism.

The results are examined on the basis of the renotrophic hypothesis of Braun Menéndez and interpreted as evidence for a possible link between renal growth and renal hypertension.

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Las citas bibliográficas se harán en el texto mediante números [por ej.: algunos autores (2, 15) y en especial Jones (5)] o autores y año [por ej.: (Breslauer, 1919)]. Al final del trabajo la bibliografía se ordenará alfabéticamente y con numeración progresiva, en el primer supuesto, y alfabéticamente en el segundo. Para las abreviaturas de las revistas, etc., se seguirán las recomendaciones del World List of Scientific Periodicals. La disposición de tales citas debe ajustarse a los ejemplos siguientes:

(1) Houssay, B. A., Lewis, J. T., Orías, O., Braun Menéndez, E., Hug, E., Foglia, V. G., Leloir, L. F.: *Fisiología Humana*, 3ª edición, El Ateneo, Buenos Aires 1954.

(2) Whittembury, G., Ramírez, M., Fernández, J., Monge, C.: *Acta physiol. lat.-amer.*, 1955, *5*, 117.

De acuerdo con el carácter del artículo (artículo de conjunto o comunicación original) contará o no el título completo de los trabajos en la bibliografía.

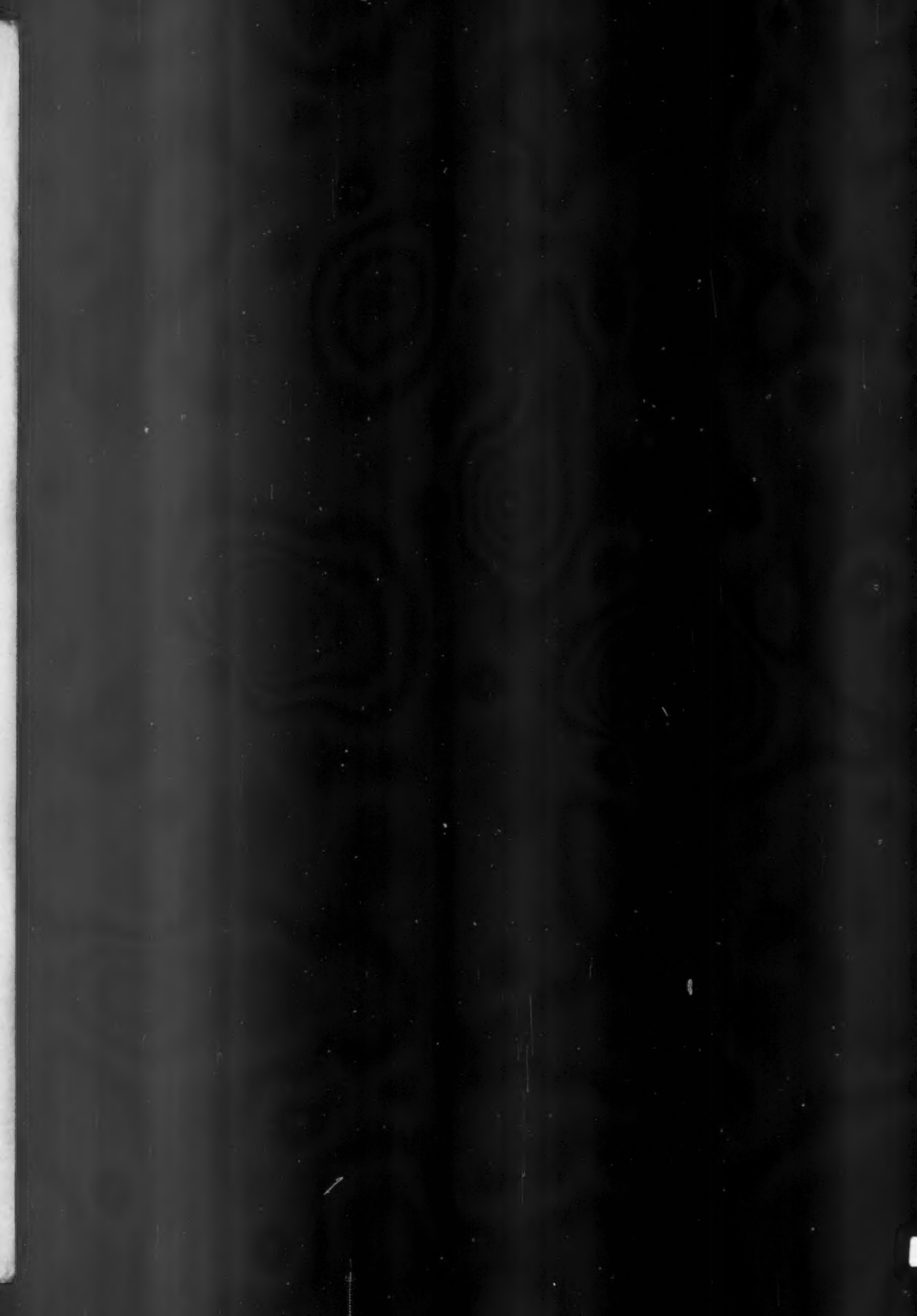
Se recomienda a los autores consultar las instrucciones aparecidas en ACTA PHYSIOLOGICA LATINOAMERICANA, 1960, Nº 2, o solicitar un apartado mediante el envío de m$n ..... (o u$s .....) a la Administradora: Srta. Josefina Yanguas, Obligado 2490, Buenos Aires, Argentina.

Las medidas y símbolos deben expresarse de acuerdo con las recomendaciones de la Comisión de Símbolos, Unidades y Nomenclatura de la Unión Internacional de Física, aprobados en Amsterdam, en junio de 1948 (*Cienc. e Invest.*, 1949, *5*, 433).

SE EXPONEN A CONTINUACION ALGUNAS ABREVIATURAS COMUNES

	Castellano	*Inglés*		*Castellano*	*Inglés*
metro	m	m	litro	l	l
centímetro	cm	cm	centímetro cúbico	cm^3	cc
milímetro	mm	mm	mililitro	ml	ml
micrón	μ	μ	kilogramo	kg	kg
milimicrón	$m\mu$	$m\mu$	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
segundo	s	sec	Microcurie	μC	μC
milisegundo	ms	msec	por ciento	%	%

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) —0.90 (no 0,90).

Corrección de pruebas: Una de las pruebas de imprenta será remitida a los autores, quienes deberán devolverlas corregidas, dentro de las 48 horas subsiguientes a su recepción. Las modificaciones fundamentales en la corrección de las pruebas en desacuerdo con los originales no serán tomadas en cuenta.

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